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THE
ANATOMICAL RECORD

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THE ANATOMICAL RECORD, VOL. 30, NO. 5

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ANOVULAR GRAAFIAN FOLLICLES IN MAMMALIAN OVARIES[1]

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TWO PLATES (EIGHTEEN FIGURES)

LITERATURE

A number of workers have more or less casually mentioned seeing in the mammalian ovary epithelial structures devoid of eggs; but we find only three authors who give them especial attention. Van Beneden ('80) describes two types of such structures in bats. The first are fragments of the superficial epithelium ('epithelial nodules') that penetrate into the stroma of the ovary and show one to several cells in cross-section. The second type consists of swellings in medullary tubes simulating follicles that have become atretic through the invasion of the epithelium by the theca.

Regaud and Lacassagne ('13) published a special study upon anovular follicles in the rabbit ovary in which they found that these "always accompany the normal Graafian follicles whose position and characteristics they share exactly." They vary from mere 'nodules' with two to five cells in section, surrounded by a basement membrane, to anovular 'vesicles' possessing somewhat of an antrum containing liquor folliculi. The two types are regarded by the authors as stages in the development of one and the same structure, arising in the first instance by fragmentation of anovular egg tubes, since they are present only in young animals and the number of anovular follicles decreases with the age of the animal.

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[1] Contribution from the University of Texas, Austin, no. 187.

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Similar anovular follicles were found in the ovary of the lemur, Galago mozambicus by Gerard ('20). This author postulates a double origin for these structures. Primary anovular follicles, the homologues of the epithelial nodules of Van Beneden and of Regaud and Lacassagne, arise directly from Pflüger's egg tubes, separating off without their compliment of oogonia. They subsequently grow by cell division, their cells arrange themselves radially, and thus the anovular vesicles of Regaud and Lacassagne are secondarily formed. A second class arises by death of the egg which is suffocated by the follicular cells and resorbed.

On the manner in which the anovular follicles disappear the three writers quoted are silent.

MATERIAL

The present study grew out of an examination of opossum ovaries prepared for other purposes, the anovular follicles having been encountered incidentally. The ovaries were prepared by various persons and by a variety of fixing and staining methods. Bouin's and Flemming's fluids were mostly employed for fixation, and Delafield's and Heidenhain's haematoxylin for staining. The ovaries of two Macacus rhesus monkeys in early pregnancy were obtained through the courtesy of Dr. N. Hollister, of Washington, D. C., and of our colleague Prof. T. S. Painter. These ovaries were excellently fixed in Bouin's. Armadillo ovaries we were privileged to examine in the collection of our colleague Mr. Deluz Hamlett. The armadillo ovaries were all taken in October when a free vesicle is to be found in the uterus.

THREE TYPES OF ANOVULAR FOLLICLES IN THE OPOSSUM

For the sake of convenience, we may classify the anovular follicles found in the opossum as primordial, medium, and mature, chiefly on the basis of size. All of them are provided with a definite, sometimes greatly accentuated basement membrane (figs. 13, 16). The theca interna in general corresponds to that of normal follicles at a similar stage of development and preservation. Occasionally the theca is found to be greatly increased in cellular elements (figs. 15, 17)—a statement that can also be made of normal or atretic follicles containing eggs.

Anovular follicles usually accompany normal ones in the same stage of development, as, for example, in figures 11 and 12. Their common origin is therefore all the more probable, and this reaches the point of certainty when they are surrounded by the same theca, as in the case of the upper pair of follicles shown in figure 11. The anovular follicles of all three classes are found at random among normal follicles of the same stage of development, not restricted to any region of the ovary. In one case (no. 593) many mature anovular vesicles were encountered near the hilus of one ovary where a remarkable number of them had been formed (fig. 11, Hartman and League, '25).

Nor are the anovular follicles limited to young animals, as Regaud and Lacassagne maintain for the rabbit. The epithelial nodules are supposed by these authors to be formed in the young animal during oogenesis, to grow during the early months or years of the animal's life, and to disappear some time after sexual maturity. In the opossum we find primordial anovular follicles in the egg nests of almost all ovaries. Larger anovular follicles may or may not be present. They are found in pregnant or non-pregnant females, in young animals or in multiparae, during the breeding season or in anoestrum, and in all stages of the oestrous cycle. There may be a few or several dozen medium and mature anovular follicles in the ovary at the same time.

Primordial anovular follicles (figs. 12 and 13) are usually of approximately the same size as primordial ova with their compliment of granulosa cells. In the latter the granulosa cells are flattened out over the surface of the ovum, while in the former they arrange themselves unhampered and loosely fill the space within the basement membrane. The nuclei appear normal and are occasionally seen to be in mitosis. In addition to these groups of granulosa cells there are smaller aggregates or 'nodules' (fig. 12) which are doubtless related to the usual type just described.

The 'medium' anovular follicles are more compact in appearance in section (figs. 3, 14, 15). Their greater size as well as compactness is due to the decidedly larger number of cells. A more or less radial arrangement is assumed by the peripheral layer of cells. Mitoses are also occasionally encountered in the medium follicles (fig. 14).

Strikingly different from these types of anovular follicles, primordial and medium, are the 'mature' follicles. While the former two classes may not readily be distinguished from tangential sections of normal follicles, the latter may seldom be thus mistaken. They are distinctive. They are always larger, usually much larger, than the medium type and average two and one-half times the diameter of the largest specimens figured by Regaud and Lacassagne. We have adopted the term 'mature' because they seem to have attained the largest possible development and are in process of disintegration. The cells are large and the cell membranes are difficult or impossible to demonstrate, so that the structures have the appearance of a syncytium. The nuclei are mostly peripherally arranged, with scattering of several nuclei near the center (figs. 10, 11, and 16). They are mostly pycnotic. Mitoses are absent. The cytoplasm is reticular or fibrillar and fails to take on any counterstain such as eosin or orange G; hence the structures stand out brightly against the stromal background. There may be an indication of an antrum with liquor folliculi in the center of the follicle.

MONKEY AND ARMADILLO

Primordial anovular follicles are to be seen in the egg nests of both Macacus rhesus and Tatusia novemcinctum. They do not differ from those described above for the opossum.

In the two monkey ovaries several 'medium' anovular follicles like that shown in figure 9 were encountered; but there were found a more considerable number of Graafian follicles in the process of becoming anovular, as will appear below.

In most of the armadillo ovaries examined there appears in varying numbers a type of anovular follicles somewhere between the 'vesicles' of Regaud and Lacassagne and the 'mature' anovulars of the opossum. They are more like the former, but are larger and have two or three rows of nuclei neatly and symmetrically arranged in radial fashion. Often the remnants of the ovum may be seen in the center, in which case the ovum is surrounded by a radially arranged layer of cells. In other cases a space appears where the egg would be expected, or the structure may consist of a solid mass of cells. In several ovaries these structures were found in larger groups throughout the cortex of the ovary, and in some groups, consisting of scores of follicles in a section, not a single follicle possesses even a remnant of an egg, though the granulosa seems entirely normal. Occasionally one encounters the exact counterpart of the 'mature' anovular follicles described above for the opossum ovary. It is very probable that these are the senile stage of the more common type and are in the process of decay.

ORIGIN

Like Gerard ('20), we find evidence pointing to two possible origins for anovular follicles.

The primordial anovular follicles are clearly derived from the same source as the normal follicles, namely, from the egg tubes of Valentin-Pflüger. In the course of their fragmentation and dispersal throughout the ovary some groups of epithelial cells were simply left without eggs—an explanation which is accepted by all of the authors cited above. It is quite possible, also, that in this process ova become separated without their epithelial partners, for we find 'naked' eggs also in the stroma of the ovary, as others, e.g., Strahl, have done before us. Some of these eggs undergo some growth before their inevitable dissolution. Nature is indeed careless in her sorting of 'royal cells,' the ova, and their 'satellites' (Gerard).

The 'medium' anovular follicles of the opossum develop in part, we believe, by cell division of the elements of the primordial epithelial groups. Regaud and Lacassagne so explain the origin of 'anovular vesicles' of the rabbit also. It is quite probable, however, that many of them, perhaps all in the armadillo, arose by the second method, now to be mentioned.

The large mature anovular vesicles of the opossum have certainly a different origin, and this is true also of practically all of the large anovular follicles of the armadillo and most of those found in the monkey. It is clear that they arise by death and resorption of the ovum or, more rarely, by the migration of the ovum out of the membrana granulosa. Both processes are shown in a large number of transitional cases.

Figures 1 to 5 show three different opossum follicles in the process of losing their ova by expulsion at an early stage of development. Figures 1 to 3 represent three sections of the same follicle. The section shown in figure 3 has already the definite appearance of a 'medium' anovular follicle. More numerous cases are, however, encountered of follicles in which the ovum is in the process of absorption, as, for example, the one shown in figure 6, in which the egg is represented by several fragments in fatty degeneration. Transitional stages in this process are exceedingly numerous in the armadillo.

In the macaques the process is essentially the same, but here the eggs usually fail to grow, while the granulosa develops normally. Thus in figures 7 and 8 are shown two such follicles, well advanced, but containing primordial ova, which are frequently moribund, as in figure 8, in which the remains of the cytoplasm takes on a dark stain. In most of these follicles the granulosa is normal except in the center, in which there is a tendency for the cell walls to break down and the cytoplasm to become very loosely reticular as in the 'mature' anovular follicles of the opossum.

In interpreting the follicles just described (figs. 7 and 8) we considered the possibility that the 'primordial ova' might be swollen granulosa cells simulating ova. We have, however, in the monkey, found no evidence for this assumption and are confident that the large cells of the follicles in question are really germ cells.

EGG DISINTEGRATES FIRST

It is thus apparent that for the cases described above the much-discussed problem as to which undergoes degeneration first, the egg or the granulosa, must be decided in favor of the egg in the cases under discussion. Of this there is no question—no room for debate: hundreds of follicles have been encountered in which the ovum is almost or entirely resorbed, but whose granulosa is absolutely normal so far as we can determine normality by microscopic examination. It seems probable that the granulosa can develop to some extent without the simulus shown by Walsh ('17) to issue from the ovum and to cause mitotic division in the granulosa. Our observations indicate, too, that Seitz's dictum, "Ohne Ei, kein Follikel," is somewhat exaggerated; it must, however, be true in the main, for eventually the anovular follicles reach the senile stage and degenerate.

FATE OF THE ANOVULAR FOLLICLES

As already indicated, anovular follicles meet the fate of all Graafian follicles except those that reach maturity and rupture: they become victims of atresia. Complete evidence of degeneration of the 'mature' anovular follicles in the opossum is found in the Flemming preparations, for in many of these typical lipolytic degeneration is apparent, as in figures 17 and 18. Figures 10 and 18 are made from preparations of the same ovary, the former being from the half fixed in Bouin's fluid, the latter from the half fixed in Flemming's fluid.

We conclude, therefore, from the foregoing evidence that, after the loss of the egg, the follicle may develop for a time, especially if the egg is lost early in the growth of the follicle. Certainly, the follicle may survive for a long time, as evidenced by the large population of anovular follicles in which the granulosa is perfectly intact as compared with the small number both of follicles found in process of becoming anovular and of degenerating or 'mature' follicles.

SUMMARY

1. Ovarian follicles devoid of eggs and possessing perfectly normal granulosa, basement membrane, and theca interna are common in the ovaries studied: opossum, monkey, and armadillo.

2. Among nests of primordial eggs are always found anovular 'primordial' follicles consisting of granulosa cells only, loosely arranged within a basement membrane. In the opossum these may occur in large numbers. They arise from Pflüger's egg tubes and are simply groups of epithelial cells that failed to be associated with the 'royal cells,' the ova (Gerard).

3. These anovular primordial follicles may grow by cell division to form the second type or 'medium' follicles, consisting of many compactly massed granulosa cells.

4. Other anovular follicles arise by the loss of the egg—a process which can readily be followed. The egg may crawl out of the granulosa, or more usually, the ovum degenerates and is resorbed in situ.

5. Follicles that thus become anovular by loss of the egg are all small structures that have not yet acquired an antrum.

6. Evidence of identity of origin of normal and anovular follicles is their association together in similar stages of development. Two follicles, an anovular and a normal, may be surrounded by the same theca interna.

7. The anovular follicles eventually undergo chromatolytic and lipolytic degeneration. But it is apparent that the granulosa is able to maintain its integrity for some time without the egg.

8. The formation of anovular follicles through loss of the egg may be considered a type of atresia. Thousands of cases in which the egg degenerates before the granulosa have been seen by the writers.

9. The list of mammals in which these structures have thus far been described now include a marsupial, an edentate, a rodent, several bats, and two primates.[2]

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[2] Since writing the above, we have found a number of 'mature' anovular follicles of the general type shown in figure 11 in the ovary of a dog, a pregnant animal from which we removed several 1-mm. blastocysts.

PLATE 1

EXPLANATION OF FIGURES

All figures except 7 to 9 concern the opossum.

1 to 3 Three sections of a young follicle in which the egg (fig. 1) is being expelled. No. 3–7. × 200.

4 To the left an anovular follicle. In center an egg with a portion of the granulosa showing as a cap above. No. 476–5. × 200.

5 A 'naked' egg (binuclear), with granulosa membrane to left. No. 496–5. × 200.

6 Follicle with remnants of ovum in center. Flemming fix. No. 582. × 50.

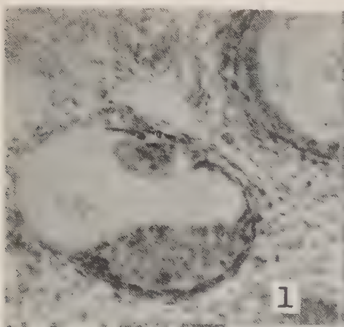
7 Well-developed follicle of Macacus rhesus, with primordial ovum. No. 2–10. × 500.

8 The same, no. 2–7. The ovum is moribund. × 500.

9 A 'medium' anovular follicle from same ovary as figures 7 and 8. × 200.

10 Four 'mature' anovular follicles. No. 593–7. × 50.

11 Three 'mature' anovular follicles and one normal follicle (*F*) with egg. The same theca (*T*) surrounds both a normal and an anovular follicle. No. 792–5. × 100.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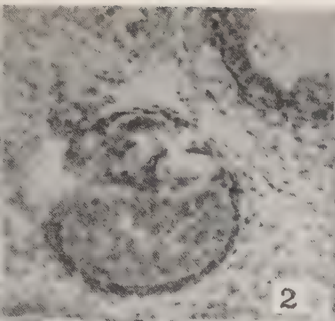
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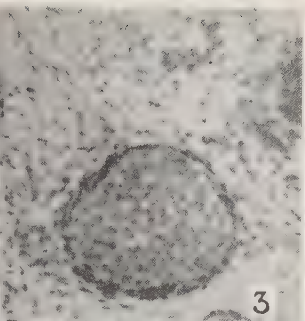
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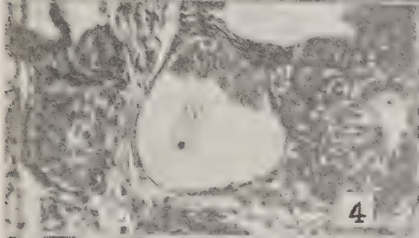
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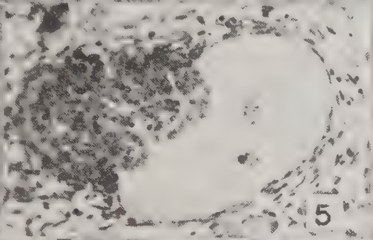
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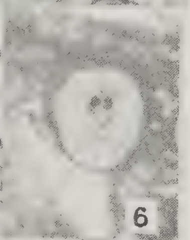
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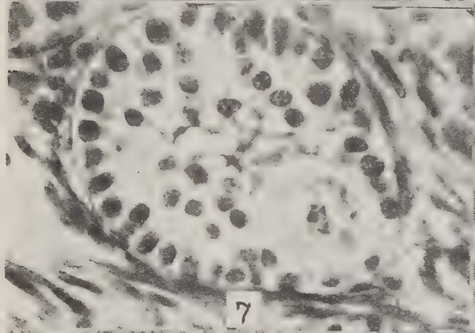
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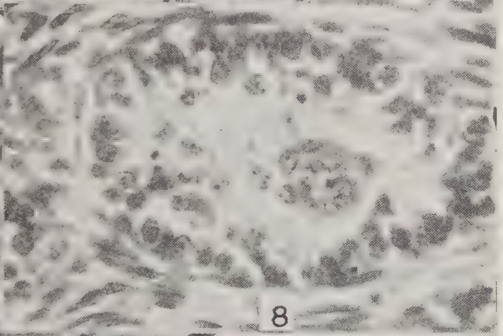
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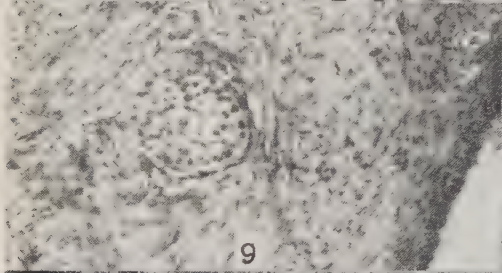
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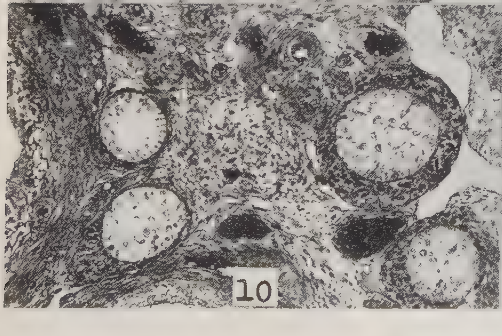
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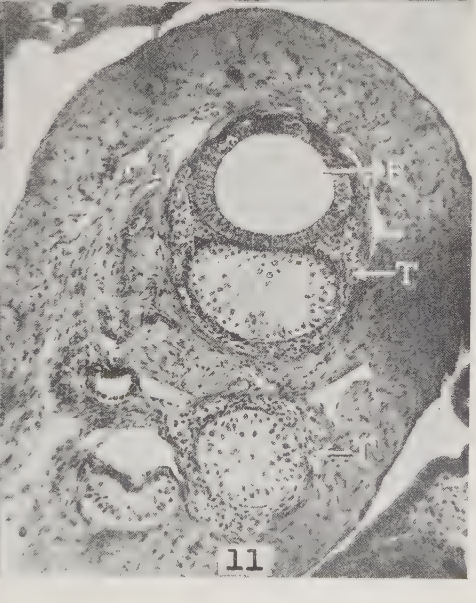
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PLATE 2

EXPLANATION OF FIGURES

All figures concern the opossum.

12 Three anovular primordial follicles (above) and four primordial ova together with several 'epithelial nodules.' The albuginea has been torn away above, placing the anovular follicles in favorable position. No. 525–7. × 500.

13 Primordial follicles, anovular and normal. Note basement membranes. No. 2–12. × 500.

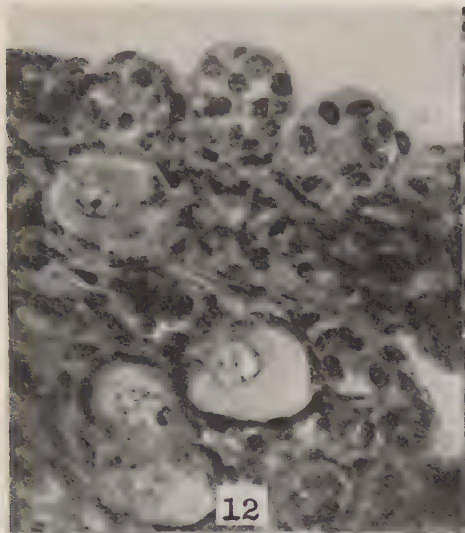
14 Three 'medium' anovular follicles and one normal follicle. Rete ovarii below. No. 908. × 100.

15 Granulosa without egg surrounded by large mass of theca which is being separated into groups of interstitial tissue. No. 819–3. × 200.

16 A 'naked' egg (center) and several degenerating or 'mature' anovular follicles, together with groups of interstitial cells. No. 608–3. × 200.

17 Anovular follicle ('mature') and portion of another, showing fatty degeneration. Flemming fix. No. 102–3. × 200.

18 The same. No. 593–7. × 100.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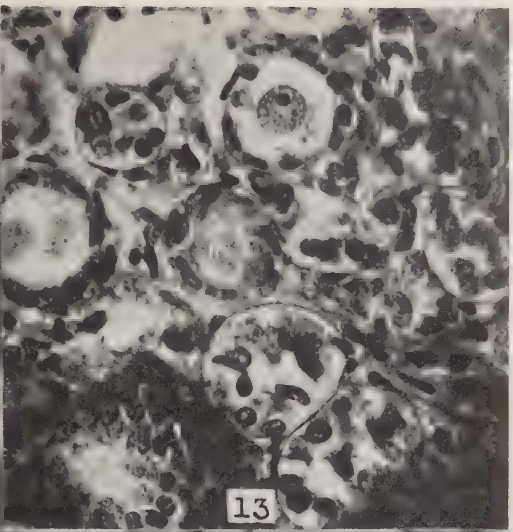
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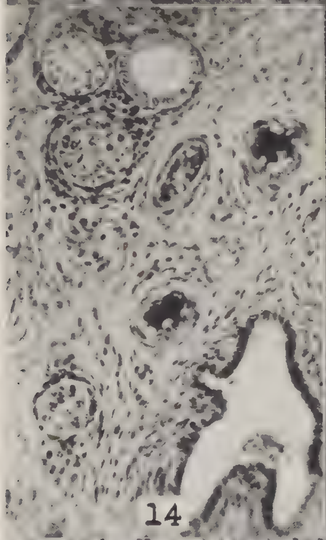
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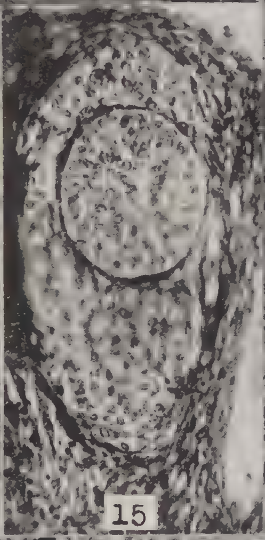
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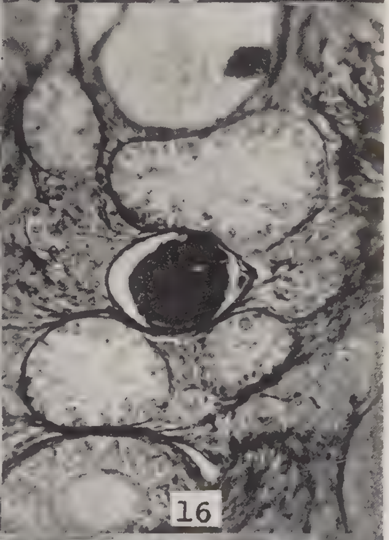
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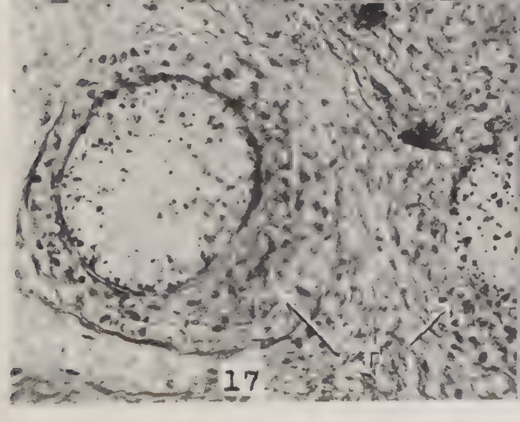
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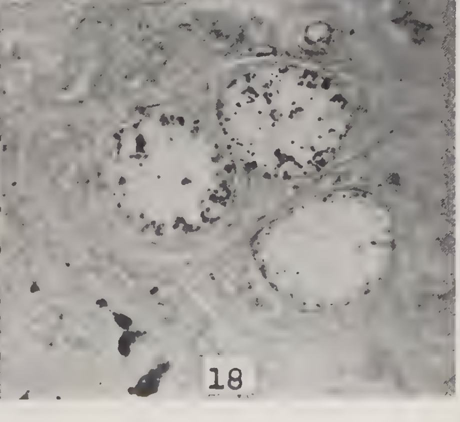
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THE ENDOTHELIAL PHAGOCYTE. A CRITICAL REVIEW

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INTRODUCTION

Foreword

The title of this review constitutes one of the many aliases assumed by a cell concerning which a great deal of work has been done and much written during the past five years. For over half a century it has been the subject of bitter controversy: sometimes considered as representing a group which comprises several distinct cell races, sometimes regarded as no group, but a single cell type capable of taking on a variety of forms. The older authorities inclined to the latter theory, but as staining technic improved and our knowledge grew, the true character of this group became, paradoxically, more uncertain and there was a tendency to consider that it might represent a number of cell types which, under certain circumstances, approached a common form. When seen in the circulation, large mononuclear cells were supposed to be produced by the bone-marrow; if they lay in the areolar tissue, they were attributed to the fibroblasts; if situated near vessels, their origin was considered to be endothelial or adventitial, as the case might be. Thus the pulmonary epithelium, the mesothelium, the omental lymphoid tissue of the 'taches laiteuses,' the lymphoid reticulum in general, and even lymphocytes have all been named as possible sites of origin.

As a result of this confusion, the nomenclature of these cells became thoroughly involved; in the circulating blood they were called 'large mononuclear leukocytes,' 'macrophages' by Metchnikoff, 'endothelial leukocytes' by Mallory, 'macrocytes' or 'monocytes' by other authors, and 'transitional cells' if their nuclei showed a tendency to approach the horseshoe form of the true transitional myelocyte. In the tissues they masqueraded under a still more imposing array of names: 'macrophages', 'wandering connective-tissue cells,' or 'adventitial cells' (Marchand); 'cellules rhagiocrines' (Renaut); 'clasmatocytes' (Ranvier); 'endothelial leukocytes,' 'polyblasts' (Maximow); 'Pyrrholzellen'

(Goldmann); 'histiocytes' (Aschoff); 'epithelioid cells,' 'dust cells,' 'Herzfehlerzellen' (shockingly mistranslated as 'heart-failure cells' in American literature) in a variety of pathological conditions; and yet others which might be added to this list. F. A. Evans ('17) has given a very clear account of the confusion surrounding the subject and listed the various 'aliases' and their respective proponents.

The reason for all this confusion depends upon our uncertainty as to the origin of the cells in question; until this is cleared up the subject must remain involved. Histologists and pathologists are fairly unanimous as to the morphology and functions of the various types, but even to-day there are warring camps that cannot agree as to their origin. It is the purpose of this review to present the various aspects of the subject in as unbiased a light as is compatible with the somewhat decided views of the writer, in an attempt to bring into one article reference to most of the researches on the origin and function of the endothelial phagocyte.

Historical

Fifty years ago, Virchow recognized the phagocyte and attributed its origin to the fixed connective tissue. Next Metchnikoff and Cohnheim advanced the theory that it was an emigrated blood leukocyte. After 1890, the impression that it was derived from fixed tissues gained ground, but the term 'fixed tissues' was applied (especially in Germany) not only to the fixed connective tissue, but to the vascular endothelium also. Thus two opposing factions came into existence: one championing the connective tissue, the other the endothelial origin of these cells. Earlier investigators soon learned that 'macrophages' would phagocytose mechanical suspensions of pigments, such as indigo, carmin, cinnabar, etc., and carried out experiments upon which much of the later work is based.

In 1904, Ribbert introduced the use of lithium carmin, a colloidal sol (which he called 'gelöstes Karmin'), as an intravenous injection for vital staining, finding that it was taken up only by those cells which comprise what is now known as 'the reticulo-endothelial system,' and by the renal and hepatic epithelium. It was not until 1914 that this method came into general use, although it stimulated experimentation with other colloidal substances and dyes. Thus Bouffard ('06) tested out various benzidin dyes and Oppenheimer ('08), at the instance of Askanazy, employed colloidal silver in the study of the histogenesis of liver tubercles. In 1909 and 1912, Goldmann published two important articles on the use of benzidin dyes for vital staining, testing out a number of these which had lately been introduced by Ehrlich and Shiga. His work was, unfortunately, so voluminous and all-embracing that much of it failed to reach an appreciative public and was, therefore, insufficiently recognized. Furthermore, it appeared in a surgical, rather than in an anatomical or pathological journal. The principles did, however, stimulate further work along the lines he laid down. Evans, Bowman, and Winternitz ('14) repeated and confirmed his experiments in connection with the vital staining of Kupffer cells and liver tubercles. Evans and Schulemann ('14) published an article on the chemistry of the benzidin group in vital staining, while Aschoff and Kiyono ('13) and Kiyono ('14) wrote articles and a monograph on the vital staining of the 'reticulo-endothelial system' with lithium carmin. To them we owe the term 'histiocyte,' for they consider that the large mononuclear phagocytes originate in the reticular tissue and have named them accordingly (*ἱστίον*, 'little net,' and *κύτος*, 'cell'). H. M. Evans ('15) calls the 'reticulo-endothelial system' the 'specific endothelia.' Between 1915 and 1919, Downey ('15, '16, '17, '18 a and b) published a very important series of papers on the action of the acid colloidal dyes administered intra vitam, with special reference to the origin and functions of the endothelial phagocyte. According to Oberling ('24), pupils of Aschoff (Katzunama, Kawamura, and Hattori) have established a relationship between the melanophores and xanthophores and the 'histiocytes.'

Meanwhile Mallory maintained the endothelial origin of the phagocyte, studied from a purely morphological standpoint. He considered that they arose from the vascular endothelium as was formerly supposed by the writers of the older German school, pointing out this fact in connection with typhoid ('98) and reiterating his contention in his text-book on pathologic histology ('14). In 1918, dissatisfied with the still uncertain status of the endothelial phagocyte and with the difficulty encountered in attempting to teach students in pathology anything authoritative about it, I began a series of investigations on the origin of this cell. Commencing with reactions to foreign substances and repeating an experiment of Forbes' ('09) done at the instance of Wolbach, the investigations were continued through 1923, benzidin dyes and McJunkin's ('18) colloidal carbon being used for the vital stains. The latter was employed because of its efficiency in marking endothelial cells in situ, in the vessel walls (McJunkin, '19), and later Higgins' American India ink was substituted because of its uniformity and convenience. Its use was recommended to me by Dr. George Wislocki, who had had favorable experience with it; just who originated its employment I do not know, except that it had been used in the Department of Anatomy at Johns Hopkins Medical School. Simultaneously, and quite independently, Permar ('20) was experimenting on the origin of the 'dust cells' of the lung, using isamin blue in his early work and later adopting Higgins' ink. Our findings were identical: that these cells originate chiefly in the vascular endothelium of the pulmonary capillaries.

In 1922, Simpson published an article from the laboratory of H. M. Evans, on the stimulation of the production of mononuclear cells in the blood by the injection of various colloidal substances, including protective colloids. She found that there were 'showers' of these cells following the injection of such substances and noted at stated intervals in the circulating blood, more copious in the pulmonary than in the systematic circulation. She divided the cells into two groups:

'histiocytes' and 'monocytes,' by means of supravital stains. Very recently Cunningham, Sabin, and Doan ('24) have concurred with this division, inasmuch as they recognize two types of mononuclear leukocyte, one originating from the vascular endothelium, the other from reticulum cells in the spleen.

Two very interesting pieces of work have been done recently by White and Smith ('24) and by Gye and Purdy ('24) in connection with the artificial stimulation of the vascular endothelium to extensive proliferation.

The former perfused the lungs of rabbits anaesthetized with paraldehyde, with Ringer-Locke solution, after injecting heparine shortly before the operation to keep the blood fluid, and after establishing artificial respiration. As soon as the blood was washed out of the circulation the fluid was continuously reperfused until it ceased to pass the capillaries any longer. It was then found that it contained a substance that, when injected into the testicle of the rabbit, would produce small nodules of endothelial phagocytes so closely packed together and so actively proliferating that the resulting picture closely simulated an endothelioma. This substance was comparatively non-toxic when injected intravenously, but nodules of endothelial cells developed in the pulmonary vessels in one case, quite similar to those seen in the testis.

Gye and Purdy have found that the continued injection of small doses of colloidal silicon dioxid will produce a tremendous overgrowth of the vascular endothelium, particularly in those portions thereof known as the 'specific endothelia.' They recognize three types of lesion: a fulminating, a subacute, and a chronic, depending upon the dosage. The second type corresponds with startling accuracy with Gaucher's splenomegaly: the spleen is greatly enlarged and innumerable swollen, vacuolated endothelial cells are found in its sinuses and in the sinusoids of the liver, the endothelium of the renal glomerular tufts sharing in the process to a noteworthy degree. Free-floating mononuclears are increased in the circulating blood, but ink injected intravenously is always taken up by the sessile cells. This probably indicates that the circulating cells are in too rapid motion to phagocytose the ink, just as in the case of the benzidin dyes, which are taken up by these cells if stagnation be produced (Downey, '17, '18). Cunningham ('22) has made similar observations, and I have noted vital staining of polymorphonuclears under like circumstances. Perhaps Fenn's ('22) theories concerning the element of chance of collision between the cells and the phagocytosed globules may have some bearing upon this subject.

A very short and inconspicuous article by Herzog ('24) appeared last spring and deserves special mention, as it proves a point long maintained on theoretical grounds by the sponsors of the colloidal-carbon method of vital staining as a means of identifying cells of endothelial orgin. He has observed the phagocytosis of India ink (type unspecified) by sessile, vascular endothelial cells in the frog's tongue in vivo; has seen these cells detach themselves from the vessel wall and become 'mononuclear leukocytes' in the circulation; and has, over a period of several days, watched other sessile endothelial cells which, after phagocytosing carbon, have detached themselves from the vessel wall and wandered through the connective tissue to distant points, carrying the ink with them. Thus he has, by actual observation in vivo, clinched the argument that, because it was based upon presumptive evidence obtained from fixed, dead material, needed just this proof to make it convincing.

Staining methods and technic

Before considering the cell under discussion in its various aspects, let us review the various methods employed in its study. The earlier investigations were carried on by means of routine stains in sectioned material. We need not consider this at length. With the advance in technic little was added to our armamentarium until the methods for staining lipins were perfected by Lorrain Smith, Ciaccio, Dietrich, and others when (by reason of their propensity for phagocytosing fatty materials) the phagocytes became of passing interest. It is just this refractoriness to special staining methods that has retarded advance in connection with this subject.

The habit of phagocytosis on the part of these cells made possible the early investigations of Slavjansky ('69) and Tchistovitch ('89), but they were unable to come to any satisfactory conclusions because of their inability satisfactorily to label the cells at their source. These investigators used intratracheal and intravenous injections of mechanical suspensions of indigo and cinnabar, Tchistovitch also using bacteria.

As has been stated, the use of colloidal stains was introduced by Ribbert ('04) and elaborated by Bouffard, Goldmann, Aschoff, Kiyono, H. M. Evans, and others. These dyes may be given intravenously, intraperitoneally, or subcutaneously in comparatively large doses, depending upon their toxicity. Trypan blue and its American analogue Niagara blue seem to be the least harmful, while Janus green is quite toxic. Lithium carmin, although non-toxic to most laboratory animals, must be used very carefully in the case of hybrid Japanese waltzing mice, judging from repeated personal experience. The colloidal metals, such as silver and gold, appear to be somewhat more specific for the liver endothelium than for that located elsewhere, but they are fair vital stains.

Colloidal carbon has the advantage of being specific for sessile endothelium; not only the 'specific endothelia,' but the vascular endothelium in various parts of the body takes it up in situ, particularly in the pulmonary capillaries and in inflamed areas. This is not the case with mechanical suspensions—a point that I have repeatedly stressed, but which is usually overlooked, as it was by Westhues ('22), who tried to repeat my experiments on the pulmonary phagocytes and failed to obtain the same results. Permar's ('23) success with this method bears out this contention. The original technic was devised by McJunkin ('18, '19) for the study of mononuclear phagocytes in human blood kept in vitro, and consisted in emulsifying a suspension of finely ground lampblack with 1 per cent gelatin. The formula of Higgins' ink is a trade secret, but the fact remains that it is a permanent colloidal suspension or sol. Thus, while benzidin dyes and lithium carmin usually stain only the 'specific endothelia,' colloidal carbon is phagocytosed by vascular endothelium outside of the reticulo-endothelial system as well and does not diffuse into the surrounding connective tissue, as do these dyes. Used in conjunction, they make an ideal combination for differentiating cells of intra- and extravascular origin.

An excellent method for attacking the problem of the origin of endothelial phagocytes is the tissue culture, originated by Harrison and perfected by Burrows, Carrel, and the Lewises (Lewis and Lewis, '24). The work of Carrel and Ebeling ('22) on pure cultures of mononuclear leukocytes illustrates what may be accomplished by these methods. Tissue cultures are, however, not the same as the living body; they lack any circulation and there is no differentiation of cells in vitro—embryonal tissue remains embryonal for years. By means of strands of fibers running from one side of the culture to the other, Burrows has produced a sort of circulation by capillary attraction, but this is a method demanding very perfect technic.

Observations carried out in vivo are, of course, the best method for deciding upon the origin of cells. Sabin's ('20, '22) work on the chick blastoderm in vitro almost amounts to in vivo observation, for a circulation is present and differentiation proceeds up to a certain point, where it is interrupted by the mechanical limitations of the culture. The investigations of the Clarks ('18 a and b, '20) on the tails of living hyla tadpoles and those of other observers upon various forms of amphibia constitute an important avenue of approach, but the results of such research must be checked up by observations carried on in warm-blooded animals in vivo, or at least by means of vital staining, before they can be accepted as applying to mammalian organisms. Thus, Herzog's work would be of interest merely to biologists studying amphibia, were it not confirmed by the study of vitally stained mammalian subjects. I understand that Professor Clark is now engaged in adapting his methods to mammalia, and the results of that undertaking will be of the greatest interest.

NORMAL OCCURRENCE AND CHARACTERISTICS OF ENDOTHELIAL PHAGOCYTES

Morphology

As seen in the blood, this cell is a rounded or ovoid structure, with a large, usually reniform nucleus and a rather prominent zone of cytoplasm. In this tissue the endothelial leukocyte and the monocyte are indistinguishable one from the other, unless a study be made of the arrangement of mitochondria and phagocytosed particles. In the monocyte there is a central area, next to the concavity of the nucleus, which contains the mitochondria and never encloses phagocytosed particles (Cunningham, Sabin, and Doan, '24), whereas the endothelial leukocyte may contain such granules in this portion of the cell. The monocyte, too, tends to phagocytose much finer particles than are commonly seen in the endothelial leukocyte (macrophage). In the case of both cell types the nucleus is reniform to ovoid, shows a delicate, rather vesicular structure, usually contains a prominent nucleolus, and varies in size in direct proportion to the size of the cell, which cannot be stated definitely, as it is very variable. In the blood stream these cells are somewhat larger than polymorphonuclear leukocytes, in the tissues they may assume gigantic proportions.

The cytoplasm is rather dense and compact in the smaller forms, reticulated or vacuolated in the larger. It contains no stainable granules, as a rule, other than phagocytosed material, if we except the mitochondria. It is said to contain no oxidase granules, but McJunkin ('22) has recently shown that this is not true and that such granules may be demonstrated in the vascular endothelium, particularly in the Kupffer cells. Owing to its powers of phagocytosis, the cytoplasm may be found to contain vacuoles of a fluid nature, fat-droplets, lipins, pigments, and a variety of other materials. It is due to this fact that it may be so satisfactorily stained intra vitam, as it takes up colloidal carbon and dyes. The exact mechanism of this process is little understood. Whether colloidal dyes are phagocytosed as globules, received into vacuoles, or taken up by preexisting cell granules for which they have an affinity, is not definitely known.

Occurrence under normal conditions

There are two forms of endothelial phagocytes: free and fixed. They are found free in the blood stream where Mallory claims that they are all of endothelial orgin, while Sabin and her adherents modify this by asserting that some are of endothelial, others of reticular parentage. There are those who deny that the endothelium plays any part in their production, naming the bone-marrow as their progenitor. Phagocytes are also found free in areolar connective tissue everywhere in the body, where they show a predilection for perivascular spaces, and they are regularly seen in the sinuses of the spleen and lymphnodes. It is probable that a few lie in the pulmonary alveoli, occupying recesses in the lining epithelium. I have called attention to this (Foot, '20 b) and two such cells are beautifully illustrated in plate IV, figure 3, of Permar's ('20) first article. A few free phagocytes may be seen in the liver sinusoids.

In their sessile form they occur in the liver as Kupffer's ('76) 'Sternzellen,' in the reticulum of the spleen and lymph nodes, as well as the larger sinuses, and (according to Lubarsch, '21) in other organs, such as the testis, pancreas, and kidney in the perivascular tissue. The sites just enumerated in the case of sessile endothelial phagocytes form the 'reticulo-endothelial system' of Aschoff. Oberling ('24), in his review of the subject, says:

> Summing up the articles just quoted, we retain the fundamental idea that there exists a well-characterized cellular system, readily demonstrated by vital staining, essentially comprising the reticular, endothelial, and histiocytic cells. This cellular complex forms a vast syncytium which sends its expansions into all parts of the organism, including the circulating blood.

This is a somewhat exaggerated statement of the creed of the 'reticulo-endothelial' school—an interesting concept, but probably too extended in its scope, which could further be expanded to include the vascular endothelium of the lung (which phagocytoses colloidal carbon with avidity) and other vascular endothelia sometimes found to contain this substance. It is wiser to preserve the old boundaries between the circulating blood and the endothelium, with the understanding that both contain closely related elements which may, under certain circumstances, become involved in the same functional processes. The osteoclast probably represents another form of endothelial phagocyte, closely resembling the Langhans giant cell, which we shall discuss later.

Physiology of the endothelial phagocyte

As to the process of phagocytosis, it would be well to refer the reader to a series of articles by Fenn ('21 a, b, c; '22 a and b), who considers the subject from many points of view, among them the temperature coefficient of phagocytosis, the chance of collision between the particles to be phagocytosed and the phagocyte, the question of stickiness, surface tension, etc. Kite and Wherry ('15) have discussed this in connection with leukocytes and bacteria, from the standpoint of stickiness.

The normal functions of phagocytes are being studied more and more and the relation of these cells to the removal of certain substances from the circulation has been investigated from various angles. Kyes ('14, '15), Cary ('15), Motohashi ('22 a), and others have concerned themselves with the removal of erythrocytes, autogenous and foreign, from the circulation by the agency of these cells in spleen and liver.

Drinker and Shaw ('21) have made a careful quantitative study of the removal of manganese from the circulation by the endothelium and of the relative importance of this tissue in the various organs involved in the process. The endothelium as a source of fibrinogen is now being investigated.

Endothelial block

Immunologists are becoming increasingly interested in the subjects of 'endothelial block,' increased and decreased endothelial permeability, etc. Of these the first may be mentioned in this paper. Oberling ('24) claims that Minkowski and Naunyn experimented with this phenomenon as early as 1886, although I cannot find any basis for this statement in their paper. The most recent paper on the subject, that of Gay and Clark ('24), quotes the work of Migay and Petroff, who found that injections of carmin prevented the subsequent absorption of colloidal iron; and of Kusnetowsky, who injected the fat of egg yolk and demonstrated that subsequent vital staining with trypan blue was impossible. Standenath ('23) finds that the injection of Schmincke's 'Flüssiges nankingsche Tusche' (Chinese ink) into the veins of rabbits fills the endothelium of various organs with ink globules, but does not prevent the formation of precipitins, increasing it rather. Antitrypsin substances are also increased in amount.

Gay and Clark believe that this experiment and one by Vanucci are based upon insufficient evidence. They have experimented with rabbits and rats saturated with trypan blue and have tested the hemolytic activity of their serum against washed-sheep corpuscles as compared with that of nonsaturated animals. They find that there is a striking diminution in the hemolytic power of the treated animals, comparison of the titers showing an average ratio of 75 : 1 in favor of the untreated controls. They conclude that hemolysin and precipitin production is almost completely prevented by 'blocking' or 'plugging' the vacuolar apparatus of the endothelium with trypan blue, and explain why this is not due to poisoning by the dye.

THE ENDOTHELIAL PHAGOCYTE IN PATHOLOGICAL CONDITIONS

Inflammation and repair

In inflammation and repair, whatever the exciting cause, this cell plays an important rôle: its function is primarily to act as a scavenger, to remove débris of all sorts from the field of action. The cells taking part in this process may originate from preexisting, free phagocytes already in the tissue (either by simple migration or migration plus proliferation) or from the multiplication of endothelial cells composing the walls of neighboring capillaries—it cannot be denied that some of them may come from the blood stream, but that many do so is unlikely. If the process be mild, those cells already present as 'adventitial cells' and 'histiocytes' may be sufficiently numerous to cope with the condition. They may multiply in situ, thus increasing the supply, but if the inflammation be severe, they are destroyed together with the surrounding tissue. New cells may migrate from neighboring healthy areas, and there is reason to believe that they do, but great demands can only be met by an additional supply furnished by the proliferation of the endothelium of nearby capillaries and, in the case of granulation tissue, by that of the newly formed capillary sprouts. My reasons for this statement are made clear in the fifth paper on endothelial reactions (Foot, '21 b). The various theories as to the origin of mononuclear phagocytes in inflammation are discussed in most text-books of pathology and are particularly well treated by Lubarsch ('23) on pages 514 to 581 of Aschoff's "Pathologische Anatomie," vol. I.

The removal of pigments, foreign bodies, etc.

It is in such pathological processes that the phagocyte comes into greatest prominence, and for this reason more has been written concerning it by pathologists than by histologists who meet it only infrequently. Perhaps the simplest process in which this cell is seen is chronic passive congestion. This condition in the lung brings about a crowding of the alveoli with cells containing pigment and, in soft-coal communities, carbon. Earlier writers ascribed the presence of these cells to the desquamation of the alveolar epithelium, and this contention was upheld as recently as 1919, when Sewell produced large numbers of intra-alveolar phagocytes experimentally and asserted their epithelial origin; and 1922, when Westhues came to the same conclusion. Permar ('20 a and b, '23 a and b) and I (Foot, '20 b, '23 a) have, I think, shown this idea to be erroneous; our articles are, in effect, experimental proof of the morphological studies of Klotz ('13, '18) and an amplification of the experiments of Haythorn ('13), who drew the same conclusions that we did. The fact that most of the free intra-alveolar phagocytes eventually contain ink, after this has been injected intravenously, makes it difficult to explain how it reaches the epithelium, while the idea that it was phagocytosed by endothelial cells in situ, later to be transported to the alveoli, is more readily acceptable. It is also difficult to account for the thoroughly 'histiocytic' behavior of these cells after they reenter the lymphatics after leaving the alveoli (as can be readily demonstrated) if they be, indeed, of epithelial origin. The pigment phagocytosed by these cells in chronic pulmonary passive congestion is largely hemosiderin.

The removal of pigment from the body is performed almost entirely by the endothelial phagocyte; in such congestions as just mentioned and in the obscure disease known as 'hemochromatosis,' experimentally produced and studied by Mallory, Parker, and Nye ('21), the sessile endothelium of the liver and other organs, and the free phagocytes as well, are heavily laden with hemosiderin and hemofuscein. After hemorrhage into various tissues it is usual to find large numbers of endothelial phagocytes at the site of bleeding, until pigmented foci are ultimately formed, as in the case of the brain, which can be seen with the naked eye. Malarial pigment is taken up by these cells in large quantities, and it is not improbable that they play a much more important part in the formation of 'lutein cells' in the ovary than has been credited to them. As already stated, the pigmented chromatophores of the subcutaneous tissue are considered as being 'histiocytes' by Aschoff's Japanese pupils.

Foreign material that is difficult of absorption always calls out a large number of phagocytes and, if these cannot remove it unaided, they fuse to form the well-known foreign-body giant cell, or syncytium. In tuberculosis these are called Langhans' giant cells. That they are formed by the fusion of endothelial phagocytes is now generally accepted, and this fusion may be demonstrated readily in tissue cultures (as was done by Lambert, '12) by introducing bits of cotton, etc. The cover-slip of the culture may also act as a foreign body. That the nuclei of the component cells may further divide is not only possible, but probable, for I have demonstrated mitotic figures in such giant cells in an experiment with silk sutures (Foot, '21 b). This is, however, exceedingly rare. Maximow ('24) has also studied these cells in tubercles formed in vitro. In the 'false epulis,' or non-neoplastic inflammatory granuloma of the gum, and many 'giant-cell sarcomata,' many of the giant cells present are probably formed in response to the presence of bone crumbs, resulting from necrosis of the affected bone, as Mallory ('14) has pointed out.

The endothelial phagocyte and microorganisms

It is well known that certain microorganisms are combated chiefly by the endothelial phagocyte and the recognition of the typical lesion of the disease in question is often due to the characteristic arrangement of these cells in definite patterns, as in tuberculosis, typhoid, etc. It was through a study of such lesions that the earlier work on these cells was accomplished. Some very tiny microorganisms provoke endothelial overgrowth and the production of phagocytes. This is true of Dermacentroxenus rickettsi, the cause of Rocky Mountain spotted fever, and of Rickettsia prowazeki, that of typhus (Wolbach, Todd, and Palfrey, '22). Trench fever, the reaction to another rickettsia, shows similar lesions. Mallory and Medlar ('20) have demonstrated a typically endothelial response to the still unknown virus of measles. Few cocci cause an endothelial reaction, but many bacilli do, especially those with lipoid envelopes, such as B. tuberculosis and B. lepræ. It is supposed that the lipins in these coatings render the bacilli similar to inert foreign bodies, and therefore too indigestible for the microphages, although the macrophages can deal with them more or less successfully. Prudden and Hodenpyl ('91) and Prudden ('91 a and b) have shown that dead tubercle bacilli will produce almost typical tubercles, demonstrating the importance of the foreign-body element in this case. We must discuss the reaction to tubercle bacilli later, in a special section of this review. B. typhosus is a well-known stimulant of endothelial proliferation, and increases the mononuclear phagocytes to such an extent that they flood certain tissues, effectually blocking capillary circulation. Mallory ('98) attributes much of the necrosis of the typhoid ulcer to this blockage and MacCallum ('03) has described such quantities of phagocytes entering the pulmonary circulation from the thoracic duct, that infarction of the smaller pulmonary arteries resulted. Miller and Pepper ('16) have observed intense phagocytosis of erythrocytes by endothelial leukocytes in the blood stream, following the injection of typhoid bacilli into rabbits. Schilling ('16) has reported an endothelial leukocytosis in small-pox, as well as in malignant endocarditis ('19).

Various yeast-like organisms and molds are capable of causing lesions typified by the presence of large numbers of endothelial phagocytes, and this is well seen in blastomycosis, where the blastomyces taken up by the cytoplasm of the phagocytes, in which it lies included in toto. In actinomycosis we often see examples of such phagocytic activity.

Most protozoa provoke endothelial reactions; leishmania in its various forms causes a very brisk endothelial proliferation, particularly in the liver and spleen, where the small non-flagellated forms of the organism are found within endothelial phagocytes, both free and sessile. Anyone studying the ulcers caused by endameba histolytica can testify to the great number of free phagocytes called out by this protozoon. Endothelial leukocytosis has been noted in malaria, where it is probably a response to the destruction of erythrocytes rather than to the organism itself.

Endothelial phagocytes as hemophages

An extended consideration of the part played by these cells in the destruction of erythrocytes, while proper to this paper, would carry us far afield. The importance of the cell in pernicious anemia and hemorrhagic icterus has been recognized by Kyes ('14), Levaditi, Cary ('15), and others, and much experimental work has been done in this connection. Kyes has found that, when there is an increased phagocytic activity on the part of the endothelium, there is increased antibody production—Cary has confirmed this. Pearce ('18) has found that injections of hemolytic serum into normal dogs are followed by intense jaundice, which is prevented by splenectomy, indicating the spleen as the chief site of hemolysis. As early as 1886, Minkowski and Naunyn experimented along similar lines on birds and mammals. Gay and Morrison ('23) have carried on investigations of antibody and antitoxin production in connection with 'clasmatocytes' in experimental streptococcus septicemia. One cannot pursue this theme further without invading the fields of serology and immunology.

Endothelial phagocytes and the lipin metabolism

There is a great increase in the number of endothelial phagocytes in connection with hypercholesterinemia, or other conditions of increased fatty metabolism. Anitschkow ('13) has produced a condition of extreme endothelial hyperplasia by feeding cholesterol to animals, while McMeans ('16) was able to bring about similar conditions by administering cholesterol in olive oil. Several authors have endeavored to connect this experimental condition with that of Gaucher's splenomegaly, but any such relationship is denied by Mandelbaum and Downey ('16 a and b) who consider the presence of lipoids incompatible with a diagnosis of that disease. Oberling ('24) has apparently overlooked this fact, or does not agree with these writers. He considers Gaucher's disease a disturbance of the lipin metabolism of the reticulo-endothelial system. The presence of collections of lipins in connective-tissue phagocytes, the xanthoma and xanthelasma, are thought by him to represent localized processes of a similar nature, in contradistinction to the generalized endothelial hyperplasia of lipemia. He also quotes Schultze and Lutz ('14) in connection with the lipemia of diabetes mellitus.

The endothelium in Gaucher's disease and in endothelioma

Gaucher's splenomegaly is an excellent example of tumor-like overgrowth of the endothelium of the spleen and liver, and of other organs rich in reticulum. It is fully discussed and ample references are cited in a very recent paper of Reuben's ('24) and an earlier report by Ladd and myself Foot and Ladd, '21), to which the reader is referred. It is not a true tumor, for it has none of the characteristics of tumors. The nearest experimental approach to the disease has been attained by Gye and Purdy ('24).

There are several types of tumor arising in the endothelial system: vascular, reticular, and meningeal. Of these the second type often shows cells strikingly similar to the endothelial phagocyte and its derivatives, particularly giant cells. Such tumors are exhaustively discussed by Ewing ('22) in his text-book on neoplasms, and one of them was described and the literature reviewed in a recent paper of mine (Foot, '24).

CONCERNING THE ORIGIN OF THE ENDOTHELIAL PHAGOCYTE

This may best be considered from the standpoint of the pathology of tuberculosis, as was recently done by Permar ('24) in a symposium on the endothelial cell presented at the last annual convention of the National Tuberculosis Association. In this disease one sees mononuclear phagocytes responding to B. tuberculosis in every tissue of the body. If the reader desires, he can find the earlier literature on the histogenesis of the tubercle listed in an article by Kockel ('96). Among the early students of this subject two groups may be recognized: the adherents of Baumgarten who sponsored the cells of the fixed tissues, including vascular endothelium, as progenitors of the 'epithelioid cell,' and Metchnikoff's school, who interpreted them as being emigrated blood leukocytes (see Kockel). Early observers considered the vascular endothelium to be largely responsible for these cells, then the fibroblastic origin became popular, and the pendulum now appears to be swinging back toward the endothelium and reticulum. There is also a tendency to regard epithelium as a possible site of origin in the case of the lung, kidney, and eye, although it appears to be waning in popularity.

Tubercles in areolar tissues

Tubercles of loose connective tissue are composed of cells arising from various sources: fibroblasts, histiocytes, adventitial cells, or polyblasts, according to the convictions of the observer. Mallory believes them to be of endothelial origin, and there are those who think that they are either of endothelial or of reticular parentage. Maximow has a theory that there are mesenchymal cells which remain embryonal and dormant throughout adult life, lying near vessels and in tissue spaces, to be stimulated into activity and differentiation by that process which we call inflammation. This is as difficult to prove as it is worthy of admiration; how to disprove it I do not know, and hence it is worth considering. In his "Referat über Entzündung," Rössle ('23) says this about it: "Maximow certainly makes a mistake in considering these as anything different from fibroblasts. The existence of two sorts of connective tissue cell is, moreover, not probable in the light of modern conceptions."

Maximow ('24) has produced tubercles in vitro and concludes that the epithelioid cells are produced by the multiplication of reticulum cells in the case of lymphoid tissue—that these hypertrophy, divide, and become transformed into large polyblasts. He thinks that the lymphocytes do the same. "Fibroblasts do not take any part in the formation of tubercles and giant cells." In discussing the rôle played by the endothelium in this process, he says:

> If we confine the use of this name only to the squamous cells lining the common blood-vessels—capillaries, arteries, veins, and heart—and the lymph vessels, it will be difficult, if not impossible, to find any real proof for their assumed participation in the production of the elements of the tubercle. . . . The endothelial cells either keep their former arrangement in tubes and produce new sprouting capillary branches, or they become transformed into strands of common fibroblasts. . . . The condition changes completely if we widen the boundaries of the endothelium idea and apply the term 'endothelium' to all squamous cells in general, lining blood or lymphsinuses of any kind, for instance the cells of the walls of the venous sinuses of the spleen, to the cells of Kupffer in the liver, to the reticular cells lining the walls of lymphnode sinuses, etc. If we choose this point of view, we shall have to look on the epithelioid and giant cells of the tubercles in the organs mentioned as products of endothelial transformation.

Thus he merely rules out the vascular endothelium in general and lays his theory open to criticism that I shall offer near the conclusion of this review.

As for myself, I feel that the subcutaneous tubercle is produced largely by emigration of detached vascular endothelial elements from neighboring capillaries which may remain intact, or may have been disrupted by the tuberculous process. One cannot exclude those cells lying in the tissue spaces, although experiments have not encouraged me to consider that they play an important part, for they are largely destroyed in the first onslaught of the infection. As to their origin, it is difficult to prove whence they come—they may be thrown out from time to time from the endothelium of the capillaries, they may come from distant reticular tissue via the blood stream, or they may have been in the tissue spaces since embryonal life, as Maximow maintains and we cannot as yet categorically deny. Suffice it to say that they react to vital stains exactly as do the cells of endothelial and reticulum-cell origin. Kockel's idea that vascular endothelium proliferates in tubercles as it would in any granulation tissue, but instead of forming vascular sprouts, forms sheets or plugs of endothelial cells, has always been very appealing. A theory is occasionally advanced that tubercles never form in vascular endothelium in situ. It may readily be disproved by anyone who cares to do so, for I have seen them in this situation not only in animal experiments, but in human material.

Liver tubercles

Experimental liver tuberculosis has proved that the tubercles in this organ originate from the vascular endothelium of the sinusoids. The researches of Goldmann; Evans, Bowman, and Winternitz; Oppenheimer ('08), and Miller ('02) stand alone and need no further commentary.

Pulmonary tubercles

In the case of the lung, the contest has been waged between proponents of the endothelial and those of the epithelial origin of the tubercles. Watanabe ('02), Sewell ('18), and Westhues ('22) advance the epithelial theory; Briscoe, quoted by Sewell, says that these cells may come from, 1) interstitial tissue near the alveolus (histiocytes); 2) the alveolar epithelium; 3) specialized epithelium not concerned with respiratory function. In 1869, Slavjansky indicated that the endothelium or the mononuclear leukocytes of the blood were the parents of such exudates into alveoli. Mallory, Klotz, Haythorn, Permar, and I have always maintained that these 'dust cells' (which are readily identified with the epithelioid cells of pulmonary tuberculosis) originate in the pulmonary endothelium.

Tuberculous serous exudates

Many authors have advanced the theory that the mesothelium contributes to the number of mononuclear phagocytes in these exudates, again readily identifiable with epithelioid cells in tuberculous peritonitis. This subject is well treated in a scholarly article by Cunningham ('22), in which he concludes that the only reaction on the part of the mesothelium to mild irritation is a swelling, desquamation, and subsequent degeneration of these cells. Although he quotes my first article to show that I am opposed to this view, the reader may convince himself that this is a misunderstanding, if he will refer to page 282 of my fourth, and page 635 of my fifth article ('21 a and b), where I indicate my hearty concurrence with it. He concludes his article with a statement to the effect that those cells which most perfectly retain the more primitive reactions of the mesoderm are the reticulum cells, which "almost certainly retain a capability of forming new cells of more specialized character"—the endothelial phagocytes, which he believes form the bulk of the cellular constituents of serous exudates. Thus he rules out vascular endothelium, fibroblasts, and mesothelium. This paper is well worth reading, not only on account of its experimental data, but also because of its careful analysis of the literature on the subject. A publication of Karsner and Swanbeck ('20) is diametrically opposed to Cunningham's theory. They conclude that the pleural 'endothelium' is the active phagocytic agency for particulate matter in the pleural sac and that pleural 'endothelial cells' can detach themselves and transport this material further. It seems possible that there may have been some confusion between pleural mesothelium and subpleural endothelium in this case. Gay and Morrison ('23), as well as Cunningham and I, believe that these cells are emigrated endothelial phagocytes (or 'clasmatocytes') which, after penetrating the mesothelium and phagocytosing the pigment, reenter the subpleural tissues, or lie in granulation tissue on the pleural surface if the injury be severe enough to produce such a reaction. Permar ('24) has treated this phase of the question at some length, describing experiments still in progress, which lead him to state that "the evidence in this work at its present stage points to the capillary endothelium as the source of the phagocytes dealing with particulate and other mildly irritating substances in the pleura."

Tubercles in lymphoid tissue

The reticular tissue as a site of origin for phagocytic cells has been sufficiently discussed in the body of this paper; that it plays a rôle of some importance cannot be denied, but my own impression, gained from the study of tubercle formation in lymphoid tissue, indicates that the vascular endothelium of the blood capillaries and lymphatics must also be taken into account. That lymphocytes play a part in the formation of phagocytic cells is extremely unlikely. Maximow and Schridde have made this claim for many years, but if it be true, how are we to explain the sudden acquisition on the part of this cell of phagocytic propensities, when we know that it has none so long as it retains the lymphocytic form or that of the plasma cell? The smallest, most compact endothelial phagocyte may be found to contain a few granules of phagocytosed material, but lymphocytes and plasma cells are always free from it. Why do lymphocytes appear at so comparatively late a stage in tubercle formation if they are active in producing the cells that compose it? The order of appearance of the cells in a tubercle is usually: polymorphonuclear neutrophiles, endothelial phagocytes, lymphocytes, plasma cells, fibroblasts—giant cells appearing coincidentally with the endothelial elements. Rössle ('23) says (in his Referat): "The rôle of the lymphocytes is, on the other hand, in ordinary inflammation, as in tuberculosis, an entirely secondary one and likewise unexplained."

Cerebral tubercles

Here the proponent of the vascular endothelial origin of the endothelial phagocyte is confronted with a quandary: there seems to be too little vascular proliferation to account for the production of the great numbers of epithelioid cells of meningeal and cerebral tubercles and exudates, they do not seem to arise from meningeal endothelium to any great extent, nor can their origin be traced to the spleen, for splenectomy does not reduce the number of cells present in the lesions (Foot, '22). These cells must be produced at some distance from the lesion and transported to the site of inflammation in the blood stream. That they are not glia cells is practically certain. As they are found in the blood of the meningeal vessels in large numbers, the chances are that they arise in the endothelium of other organs.

ENDOTHELIAL PHAGOCYTES AND RETICULUM FIBERS

Under ordinary circumstances free endothelial phagocytes produce no fibers, but in inflammation, particularly tuberculous, when they are banded together to form a tissue complex, they form reticulum which is readily demonstrated. This has been discussed at length by Miller ('23) in a beautifully illustrated paper. Tubercles in cerebral tissue, which is devoid of reticulum, stand out in bold relief when impregnated by the Bielschowsky-Maresch technic, because of the dense reticulum formed in them through the agency of the epithelioid cells, and this reticulum may become converted into collagenous fibers with the lapse of time. I have referred to this in several of the experiments on tuberculosis in the rabbit.

White and Smith ('24), in a paper that will appear shortly, hint that fibrin may be a forerunner of reticulum fibers. From experiments carried on last spring and not yet complete, I have concluded that the older idea—that reticulum is formed by the cells themselves—is the correct one. Producing tubercles and following the development of reticulin fibrils by means of Bielschowsky impregnation and that of fibrin by the Gram stain, I could trace no parallel between the two; they appear to be formed quite independently of one another, reticulin often preceding fibrin in its order of appearance.

That the endothelium, taken in the wide sense of that term, may form reticulum has been known since Mall's first papers (Mall '91, '96). Rössle and his pupils (Rössle and Yoshida, '09; Kon, '08, and Russakoff, '09) have demonstrated this function in cells hitherto considered as purely vascular endothelium. They have published a series of careful investigations on the reticulum of the liver, lymph nodes, pancreas and kidney, and of other organs, carried out under normal and pathological conditions. Neuber ('12) has studied the reticulum of the heart. Russakoff ('09) has also investigated tubercle reticulum, his findings coinciding with Miller's ('23). Corner ('20) has pointed out the almost universal association of vascular endothelium and reticulin fibrils and concludes that a reticulum is regularly produced by this tissue, as he can always demonstrate capillaries in close proximity to reticular complexes, with one exception: fibroblasts appear to be capable of producing reticulin under certain conditions, particularly in the medullary rays of the kidney. He says:

> For the present we can merely say that in certain organs, where true connective tissue is absent, and the blood-capillaries come into direct contact with actively secreting epithelial cells, the capillary endothelial cells themselves are able to lay down the supporting framework of the gland. Where the support of tissues is provided by fibroblasts and their products, the reticulum seems devoid of reticulum fibrils. It will be important to determine next, if possible, whether there are chemical differences between endothelial cell and fibroblastic reticulum and whether the endothelial cells which can produce fibrils are otherwise different from those which do not possess such a function.

The antagonists of this theory refute it by a claim that the reticulum is formed by fibroblasts so closely apposed to the outside of the capillaries that they are overlooked. I have never been able to satisfy myself that this is so, whereas it has always been easy, in appropriately impregnated sections, to convince myself of the validity of Corner's claims, which are further substantiated by the work of Rössle and his pupils.

RETICULO-ENDOTHELIUM AND VASCULAR ENDOTHELIUM

If the foregoing be true, where are we to draw the line between 'reticulo-endothelium' and vascular endothelium? In what respect do they differ? Both form reticulin fibers, one constitutes an anastomosing network of cells, the other vascular tubes, or the lining thereof. Do the reticulum cells, in forming the walls of lymph and blood sinuses, differ enough from vascular endothelium to justify so sharp a distinction between them?

Let us grant that there are tinctorial differences, as claimed by Downey ('22) and others, but with a like embryological origin, a similar mechanical function, the common characteristic of forming fibrils demonstrable by the same methods, and that of phagocytosis, why set up such definite boundaries? Furthermore, if we extirpate the spleen, the liver compensates for the lack of hemophages. Therefore, the claim is made that the sinusoidal endothelium is 'reticulo-endothelium,' and it is accepted. But I have shown that the pulmonary endothelium compensates for the lack of splenic phagocytes by taking up vastly increased amounts of Higgins' ink after splenectomy (Foot, '23). Are we to add the pulmonary endothelium to the 'reticulo-endothelial system' for that reason? Would it not be better to lump the entire endothelial system into one large group, differentiating between vascular and reticular cells on a morphological basis, as two great subdivisions? It would appear unwise to try to separate the free derivatives of these two groups of closely related cells, for they could readily become dedifferentiated and act as 'endothelial phagocytes,' the question of their origin from one or the other group being purely academic: probably this process depends largely upon local needs and conditions. It is here that Maximow's statement, already quoted, may be questioned: unless the vascular endothelium is totally different from the reticulum cells, there can be no sharp division between the two, nor between their derivatives. It is not to be inferred that I deprecate the advisability of making a distinction between vascular endothelium and 'reticulo-endothelium' as morphological units, it is as necessary as is that between squamous and cylindrical epithelium. Io do not believe, however, that these tissue groups should be considered as being possessed of an irrevocable specificity.

ENDOTHELIAL CELLS AND FIBROBLASTS

Maximow ('24) describes the conversion of vascular endothelium into fibroblasts in a statement already quoted, and adds that this corresponds with his findings of twenty-two years ago. Carrel and Ebeling ('22) have further substantiated this theory by observations made in vitro with pure cultures of 'large mononuclears.' I, too, have maintained the possibility of such a transformation, making statements to that effect in my fifth ('21 b) paper. In 1912 I made cultures of aseptic chicken blood clot in vitro and noted the same change from large mononuclears to forms that could not be distinguished from fibroblasts. As I could never demonstrate either fibroglia or collagen fibers, I hesitated to put forth a claim that these cells were, indeed, converted into fibroblasts. Carrel and Ebeling, with their perfected technic, have carried the experiment far beyond the limits of my early attempt. Maximow ('24) and Cunningham ('22) both maintain that there is no dedifferentiation of the fibroblast to form an ameboid phagocyte, and although I agree with them in this theory, I feel that it must be substantiated by very material evidence.

A bond of similarity that connects the endothelium and the fibroblast even more closely is the constantly recurring statement that, under pathological conditions, the reticulin fibrils may be converted into collagen fibrils (Rössle et al.). Thus we have the vascular endothelial cell (Corner, '20), the Kupffer cell, the reticulum cell, and the fibroblast all described as producers of collagen under certain circumstances.

CONCLUDING REMARKS

In conclusion it might be well to present a personal 'confession of faith' regarding the endothelial phagocyte. As to its origin: undoubtedly a descendant of the mesoderm, through its large subdivision the mesenchyma, this cell is closely related to all the mesenchymal derivatives. Normally, in adult tissue, the origin of the free phagocyte is well-nigh impossible to prove. I have already indicated the possible sources earlier in this review. Under pathological conditions, the origin of the majority of these cells from near-by vascular endothelium becomes more and more probable, judging from criteria afforded by studies carried on with intravascular injections of colloidal carbon. That phagocytes are not transported from distant centers of production in the lymphoid apparatus cannot be denied, nor can the migration of free phagocytes from neighboring areas of areolar tissue be excluded; but it appears to me that only a minority of them arise in this manner in most cases of inflammation, the majority coming rather from the neighboring capillary endothelium.

It seems certain that these cells perform phagocytic functions, lay down fibrils of reticulin and collagen, and transform themselves into giant cells, or syncytia, under pathological conditions. Probably the entire endothelium, considered in the freest sense of the term, presents a persisting mesenchymal, embryonoid tissue capable of extensive differentiation or dedifferentiation, without which the body would be incapable of repairing the ravages of injury and disease. Cunningham ('22) discusses this concept, which has been advanced by a number of writers in connection with various subdivisions of the 'reticulo-endothelial system.'

The term 'endothelial phagocyte' has been employed throughout this paper because 'endothelial' infers a cell of endothelial origin and 'phagocyte' connotes its chief function. Mallory's name 'endothelial leukocyte' infers a circulatory origin. Maximow's 'polyblast' is an ideal designation, but has already been so definitely connected with his theories as to its origin and particularly to its relation to the lymphocytes, that its general use is confusing. If its sponsor would forego any claims as to the relationship of the polyblast to the lymphocyte, a better term could not be had. 'Clasmatocyte' is a poor designation—κλάσμα means 'a piece broken off' and κύτος denotes 'a cell'—invented by Ranvier, because these cells, in amphibia, break off bits of their cytoplasm, which becomes free. 'Histiocyte' limits the origin of the cell to the reticular tissues and excludes the vascular endothelium. The term that I have used throughout denotes the origin of the cell anywhere in the endothelium and its chief function in the tissues. A critical reader may be led to suppose, from this review, that the writer is receding from a position supposedly taken in his series of papers on endothelial reaction—that the endothelial phagocyte is of exclusively vascular origin. That position has never been maintained, however, for the statements made to that effect are always qualified. At the same time, the importance of the vascular origin of these cells, particularly in pathological conditions, cannot be underestimated, and the chief purpose of my papers has always been to emphasize this function of the capillary endothelium, rather than to claim that it is responsible for all the endothelial phagocytes.

Dr. Howard H. Permar, of Pittsburgh, Pennsylvania, has been so kind as to read through and criticise the first draft of this review and to express himself as being heartily in accord with the principles herein stated and with my point of view. I am much indebted to him for this service and for many valuable suggestions.[1]

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[1] Since submitting the above for publication, two important contributions to the subject have appeared: Professor Aschoff ('24) has published his series of lectures, recently delivered here in America, the first of which is his Janeway lecture on the reticulo-endothelial system, and Professor Maximow's ('24) paper (which, in a somewhat abbreviated form, was a part of the symposium on blood, presented by the American Association of Anatomists, in Buffalo, last

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spring) has come out in full. As both of these articles are reviews, there is no need for extended quotation, but a few points should be cited.

Aschoff's lecture presents the subject from the standpoint of German investigations and covers it with characteristic thoroughness and lucidity. He discusses all the subjects we have just reviewed, taking up the relationship between the reticulo-endothelium and iron metabolism in addition.

In speaking of the 'blood histiocyte,' he stresses the transitory existence of these cells, referring to Kiyono's work in this connection and pointing out that they are produced in large numbers only to be retired from the circulation after a comparatively brief intravascular career. He says, merely, that they are 'filtered out' in the pulmonary capillaries and undergo rapid disintegration in the left side of the heart. He does not say what becomes of them after they are filtered out, whether they penetrate the capillary wall and gain entrance to the lymphatics or penetrate farther to the alveolar sacs. He suggests that they may form part of the exudate in tuberculous pneumonia.

As to the origin of 'dust-cells' in the lung, however, he indicates that Haythorn's and my work on this subject are inconclusive, and he does not mention Permar's important contributions at all. He thinks that Westhues and Sacks have clearly demonstrated that these cells are desquamated epithelium, but shows in the following sentences that these investigators employed methods so different from ours that they are not comparable. He states that phagocytes containing ink that has been injected intravenously are never found in the alveolar spaces, but as Permar and I have both maintained that the contrary is true and given ample illustrative proof, this merely reopens the controversy.

He does not believe that lymphocytes are necessarily derived from, or give rise to, reticulum cells and also points out that the so-called 'germinal centers' of lymph nodules are not what that term implies, but rather centers for reticulum-cell proliferation and for the elaboration of antitoxins, etc. He quotes Hellmann's work in this connection. Any one who has studied the pathology of the lymph nodes in acute systemic infections will agree with him heartily and appreciate his giving the subject this much-needed publicity.

Another of his statements is very interesting; he says: "I am wholly in accord with Downey, who has recently made a careful study of this problem, that reticulum cells and endothelial cells are one and the same type of cells."

Maximow's ('24) article is concerned with the hematologist's standpoint and is comprehensive and interesting. In it he gives a detailed description and a graphic diagram of the genealogy of the various derivatives of the primitive mesenchymal cell, seen from his point of view; thus affording the reader ample opportunity to understand and judge his theories on that subject.

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THE INTERATRIAL SEPTUM OF THE CHICK HEART

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SIX FIGURES

One of the striking features in the development of the four-chambered heart in birds and mammals is the maintenance of an interatrial communication throughout embryonic life. Very early in development the partitioning of the atrium into separate right and left chambers starts as if it were going to progress immediately to completion, only to be interrupted by the establishment of secondary communicating openings in the part of the septum already formed. The primary functional significance of this fact would seem quite obvious. Except for the insignificant trickle of blood from the developing pulmonary veins, there is no blood entering the left side of the embryonic heart. Were it not for the secondary opening established in the interatrial septum, the left side of the heart would be cut off with practically no blood to pump until it was, at the termination of embryonic life, suddenly flooded with the full blood stream from the newly functional lungs.

In the light of what is known of cardiac physiology, it is difficult to conceive of such a sudden increase in load being borne successfully by heart muscle which had developed practically without functional activity. The temporary short cut which part of the blood stream takes from the right atrium through the interatrial foramen to the left side of the heart effectively equalizes the functional activity of the two sides of the heart during development, but it brings in its wake other functional exigencies. During contraction of the left atrium return flow of blood through the interatrial foramen to the right atrium must be prevented. When the blood is abruptly rerouted at the end of embryonic life, this opening must be promptly closed.

In the case of mammals there is abundant morphological evidence indicating the manner in which these problems are met. The interrelations of septum I and septum II are such that an efficient valvular mechanism exists at the foramen ovale.[1] This mechanism is believed to operate in such a manner that regurgitation of blood during atrial systole is prevented. The same mechanism effects a prompt functional closure of the foramen ovale at the time of birth, pending its gradual and frequently long-delayed structural closure (Nañagas, '21; Scammon and Norris, '18). In the bird heart, however, but one interatrial septum is formed (Lindes, '65; Masius, '89; Greil, '06; Hochstetter, '06; Lillie, '08). There can be no possibility, therefore, of a valvular mechanism at the interatrial communication in the bird heart which in any way resembles that in the mammalian heart. As far as I have been able to ascertain, no conditions have heretofore been noted which in any way meet this apparent functional deficiency—a deficiency which is all the more striking in view of the elaborate mechanism existing at this point in the mammalian heart.

In reworking certain phases of heart development in the chick I was impressed by structural characteristics of the interatrial septum which have either been overlooked altogether or passed by as of no significance. Instead of being

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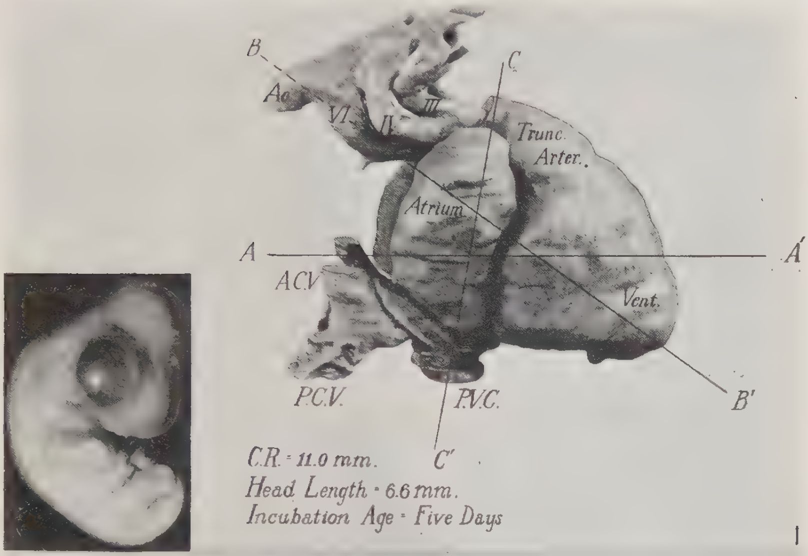
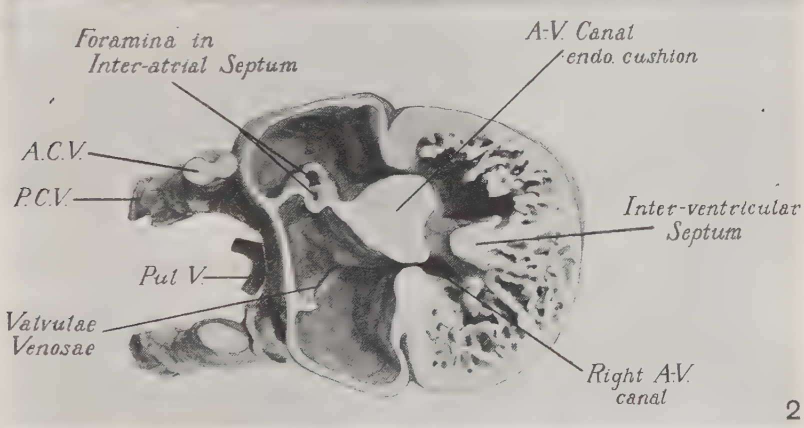
[1] The conception of a valvular mechanism at the foramen ovale dates back at least to the second century, for it is fairly accurately described by Galen. Harvey (1628) describes both its action in the foetus and its method of closure at the time of birth (Pohlman, '09). While the valvular nature of the mechanism was clearly grasped by these early workers, the information then current on the circulatory system as a whole was too meager to permit of a full appreciation of its significance. Little, if any, knowledge then existed as to the origin of structures involved. Among the more important recent papers dealing with the details of the development and interrelations of septum I and II in various mammals are those of Born ('89), Favaro ('13), Morrill ('16), and Tandler ('12).


B
Ao
VI
IV
III
C
Trunc. Arter.
Atrium
A
A'
A.C.V.
Vent.
P.C.V.
P.V.C.
B'
C'
C.R. = 11.0 mm.
Head Length = 6.6 mm.
Incubation Age = Five Days

1


Foramina in Inter-atrial Septum
A-V. Canal endo. cushion
A.C.V.
P.C.V.
Inter-ventricular Septum
Pul. V.
Valvulae Venosae
Right A-V. canal

2

Fig. 1 Dextral view of reconstruction of chick heart (model by Mitchell and Patten, × 78). The lines indicate the planes of section depicted in the following figures. The insert in the lower left-hand corner gives the record of the embryo sectioned for the reconstruction. *A.C.V.*, anterior cardinal vein; *Ao.*, dorsal aorta; *P.V.C.*, posterior vena cava; *P.C.V.*, posterior cardinal vein. Aortic arches III, IV, and VI are indicated by Roman numerals.

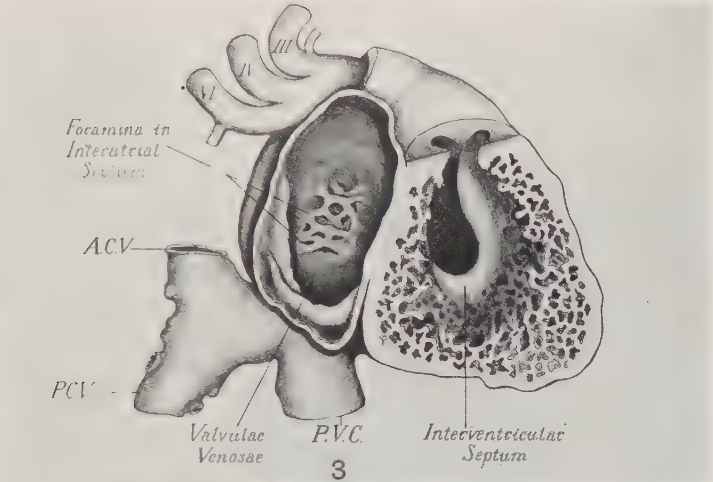
Fig. 2 Photograph of the interior of the caudal part of the heart (reconstruction by Mitchell and Patten, × 78). The plane in which the model was separated to obtain this view is indicated by the line *A–A'* on figure 1. *A.C.V.*, anterior cardinal vein; *A.–V. Canal endo. cushion,* endocardial cushion of the atrioventricular canal; *P.C.V.*, posterior cardinal vein; *Pul.V.*, pulmonary veins.

tightly stretched, as usually depicted, the interatrial septum is full and flaccid. Instead of a few circular openings which look as if they had been cut out with a punch, the openings are numerous and irregular. Frequently they are mere slits (fig. 3). Moreover, the strands of septal tissue between the foramina are not confined to a single plane, but overlap each other (figs. 2 and 4). These conditions appear to afford a structural basis for efficient valvular action. That this has not hitherto been suspected is probably due to the fact that the mechanism involved is so totally different in both structure and action from the familiar flap-like or pocket-shaped valves. While the evidence is as yet only morphological,[2] it strongly suggests the following interpretation of the functional significance of these peculiarities of the interatrial septum.

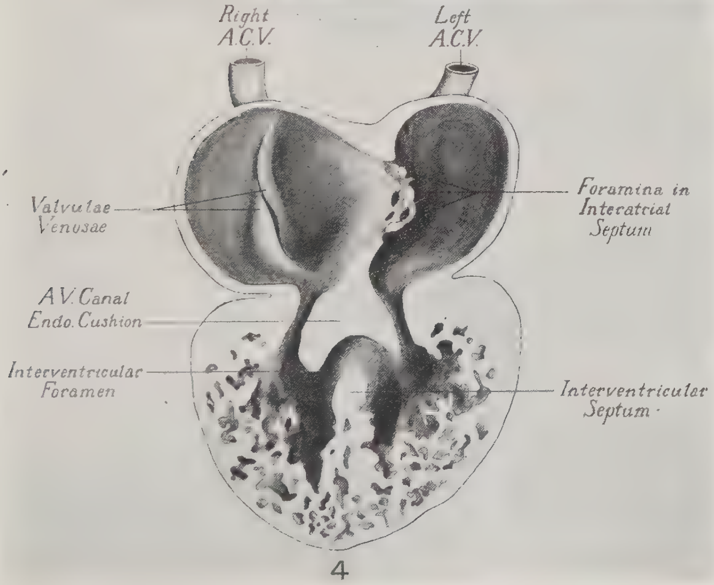
While the atria are being filled, a strong current of the entering blood is directed by the valvulae venosae toward the interatrial septum.[3] This tends to force the flaccid septum toward the left atrium (figs. 4 and 5). A reconstruction made from a heart which happened to be fixed in favorable condition shows the septum bulging into the left atrium with all its small foramina stretched wide open (fig. 2). When the atria contract, the resultant diminution in atrial size increases the fullness of the septum. At the same time the valvulae

[2] The results of this work have already been withheld for some time in the hope that experimental evidence might be obtained in addition. The uncertainty of surmounting within any reasonable time the technical difficulties involved in such a problem has made it seem advisable to present the morphological findings for their own interest. In default of physiological evidence, the suggested interpretation of the functional significance of the structures described must be regarded as tentative. As a matter of fact, most of our present interpretations of embryonic heart function are based upon deductions from structural conditions as yet unsupported by anything except fragmentary experimental evidence.

[3] Whether the blood streams entering the right atrium by way of the anterior and the posterior venae cavae pass each other without appreciable mingling or whether they become mixed in the right atrium (Pohlman, '09) has no bearing on the present discussion. The configuration of the right atrium and the course of blood through the heart are such that there would seem no room to doubt the existence of a blood stream impinging on the interatrial septum. If the existence of any such current is accepted, it makes no difference in this interpretation of the action of the interatrial septum whether it be mixed or otherwise.


III
IV
VI
Foramina in Interatrial Septum
A.C.V.
P.C.V.
Valvulae Venosae
P.V.C.
Interventricular Septum

3


Right A.C.V.
Left A.C.V.
Valvulae Venosae
Foramina in Interatrial Septum
A.V. Canal Endo. Cushion
Interventricular Foramen
Interventricular Septum

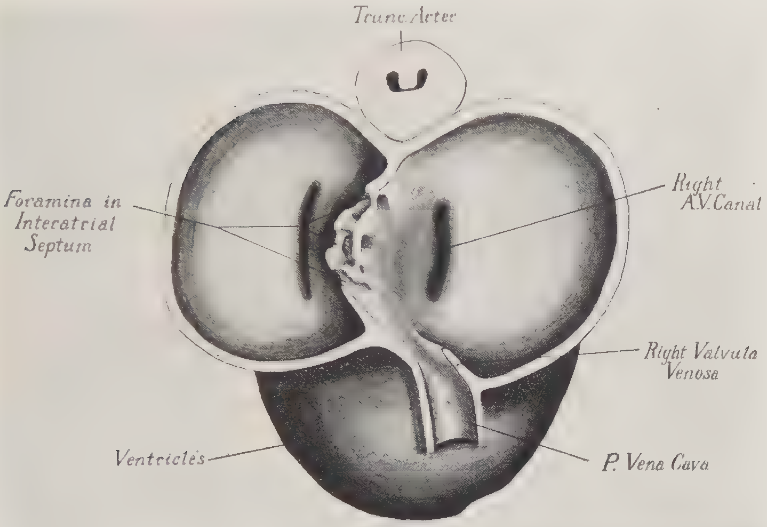
4

Fig. 3 Dextral view of heart with part of the walls removed. The right atrium and the right ventricle have been exposed by a parasagittal cut, which is sufficiently to the right of the midline to leave the interatrial and interventricular septa and the valvulae venosae intact. Note especially the irregular slit-like character of the interatrial foramina. Abbreviations as in figure 1.

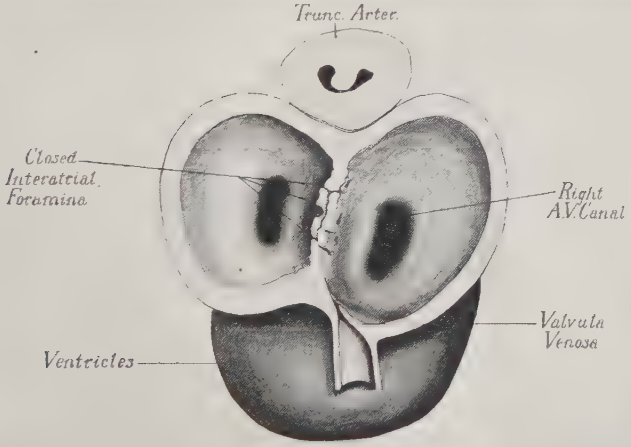
Fig. 4 Interior view of dorsocaudal part of a heart cut open in the plane indicated by *B–B′* in figure 1. The reconstruction from which this figure was drawn showed the interatrial septum, as depicted here, bulging far into the left atrium with its several small foramina fully open. Abbreviations as in previous figures.

venosae are closed and the current of blood, which during diastole was directed against the septum, ceases. Coincidently, pressure on opposite sides of the septum would, with the simultaneous contraction of the two atria, become approximately equal. These factors would tend to force the interatrial septum back into a median position and flatten it (fig. 6). The increased fullness of the septum, when it is in this position, would tend to reduce the apertures of the foramina. At the same time the overlapping strands of interforaminal septal tissue would be crowded against one another. The combined result would be a practical obliteration of the small foramina which were open when, during diastole, the septum was bulged toward the left atrium (cf. figs. 5 and 6). Under these conditions, the septum would offer an effective bar to any appreciable backflow of blood from the left into the right atrium.

The same mechanism would be equally effective in facilitating the changes incident to the rerouting of the blood at the time of hatching. During the last day or two before hatching the lungs are beginning to become functional. The concomitant increase in pulmonary circulation increases the volume of blood entering the left atrium through the pulmonary veins. The corresponding decrease in allantoic circulation reduces the volume of blood entering the right atrium by way of the postcava. Both these changes tend toward an equalization in the volume of blood entering the right and left atria and a consequent reduction in the volume of blood passing through the interatrial foramina. Equalization of the volume of blood entering the right and left atria is completely realized when at the time of hatching the extra-embryonic allantoic circulation ceases altogether. Under approximately equal pressure from either atrium, the septum would tend to maintain the more or less median position in which the foramina would be closed. The resultant approximation of the margins of the foramina would facilitate the fusions which result in the final obliteration of the communicating openings and the establishment of the imperforate interatrial septum characteristic of the adult heart.


Trunc. Arter.
Foramina in Interatrial Septum
Right A.V. Canal
Right Valvula Venosa
Ventricles
P. Vena Cava

5


Trunc. Arter.
Closed Interatrial Foramina
Right A.V. Canal
Valvula Venosa
Ventricles

6

Fig. 5 Schematic view looking from behind into the atria of a heart opened along the line *C–C′* in figure 1. This figure shows the interatrial septum stretched with its foramina open in the condition believed to exist as the atria are being filled.

Fig. 6 Schematic view of heart opened in same plane as in figure 5, but indicating the conditions believed to exist during atrial systole with the interatrial septum in the median position and with the foramina closed because of the fullness of the septum.

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NOTE ON THE PARTIAL REGENERATION OF THE CAUDAL REGION OF SPHENODON PUNCTATUM

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FOUR FIGURES

Of the five specimens of this species brought to America by Prof. C. C. Nutting in 1922, four had lost and regenerated a portion of the caudal region of the body amounting to about twelve or fourteen segments. One of the four is still alive, the skeleton of another has been prepared and mounted, and the two remaining specimens have been used for myological and histological studies. During the course of the dissection of the muscles it was discovered that the regenerated portion presented striking differences from the remainder of the caudal body region. In the living specimen the regenerated portion may be distinguished by its markedly lighter color, small size of the scales, tautness of the skin, and firmness to the touch as compared to the rest of the body.

With the dissection of the caudal region, other striking differences presented themselves. Over most of the normal portion of the body the skin is stretched quite loosely and is separated from the muscles beneath by a thin fascia;[1] in the regenerated region it adheres firmly to the underlying tissues. After deeper dissection, the reason for this superficial distinction became obvious. The major part of the regenerated region was not composed of muscle fibers at all. This discovery roused curiosity as to the exact histological composition.

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[1] The dorsal midline, the region covering the gastralea, and the region of attachment of the sphincter colli muscles are exceptions.

In the meantime, the skeleton above mentioned was prepared. The central axis was found to consist of a straight, unsegmented rod of cartilage, continuous with the last normal vertebra. Examination of the other two available specimens showed that the same condition existed in them. In fact, there were no myocommas nor any other evidences of segmentation. On summation of the anatomical anomalies of the regenerated portions of these specimens, it was decided that, as far as possible, histological studies of the material should be made. Specimen A had been fixed in chrom-aceto-formaldehyde and preserved in 80 per cent alcohol; specimen B, in 10 per cent formaldehyde. Blocks of the regenerated material from both specimens were dehydrated with alcohol and cleared in xylol. The material was too hard to section by means of a microtome, so free-hand sections were cut, stained with eosin, and mounted in balsam.

Cross-section through regenerated portion of specimen A (figs. 1, 2, and 4).

Epithelium. The apparent peculiarities of the skin have already been mentioned.

Superficial layer. This layer is composed of areolar connective tissue, most of which is white fibrous. There is no break between it and the dermis. Scattered just beneath the epithelium are a great many large pigment cells (fig. 4, B).

Deeper layer. This layer is quite vascular. It, too, is composed largely of areolar connective tissue, but contains a lesser percentage of white fibers. A number of fascicles of muscle fibers are scattered through this layer, mostly in its central portion. Pigment cells are scattered all through this layer, but are especially numerous near the central axis.

Central axis. The central axis consists of an oval tube of hyaline cartilage. This cartilage is very cellular, greatly resembling embryonal cartilage. Three series of calcification centers are present, one dorsal and two lateral. The cavity of the tube contains a granular matrix in which are embedded many very large pigment cells.

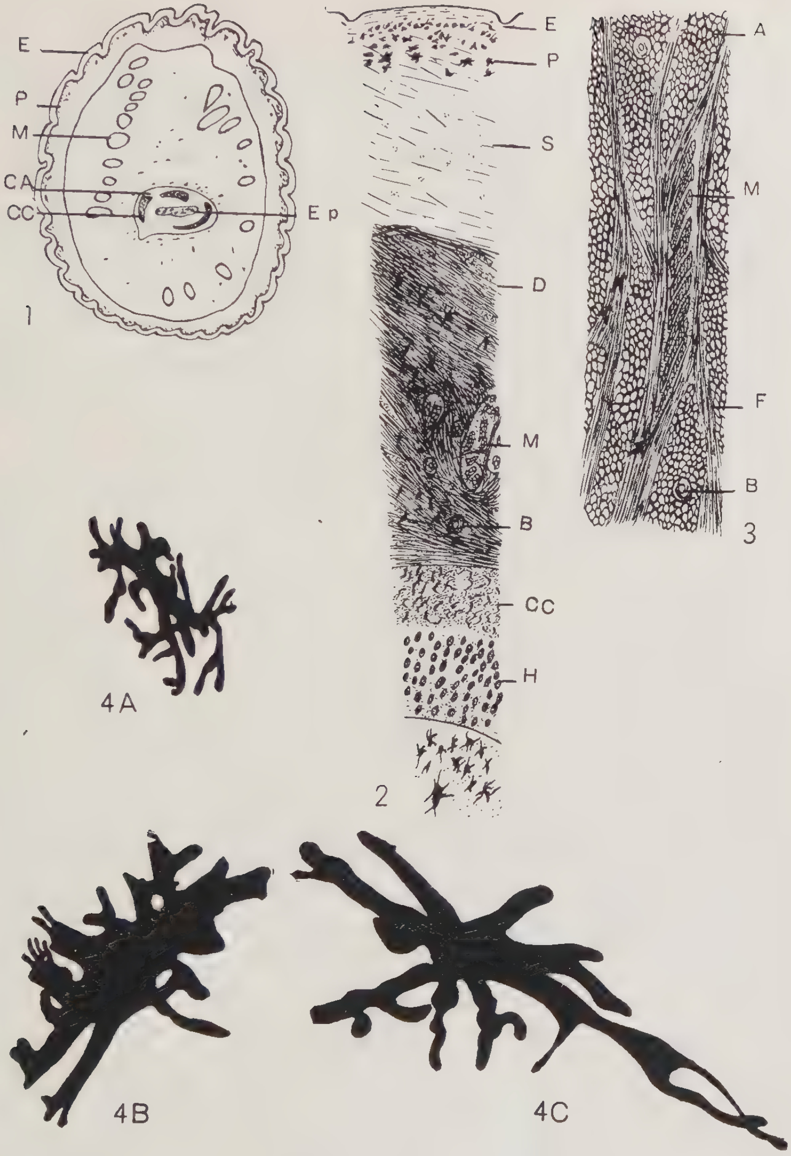

Fig. 1 Cross-section of regenerated region, specimen A.

Fig. 2 Detailed portion of figure 1. × 75.

Fig. 3 Detailed portion cross-section specimen B. × 75.

Fig. 4 A, pigment cell from the Malpighian layer; B, pigment cell from the superficial layer; C, pigment cell from the central cavity. *A.*, adipose tissue; *B.*, blood vessel; *CA.*, central axis; *CC.*, calcification center; *D.*, deeper layer; *E.*, epithelium; *Ep.*, ependyma; *F.*, fibrous connective tissue; *H.*, hyaline cartilage; *M.*, muscle bundles; *P.*, pigment cells; *S.*, superficial layer.

The cross-sections from specimen B, figure 3, show no differentiation into superficial and deeper layers, nor do they show so many scattered pigment cells. The skin and central axis are practically the same as in specimen A. The tissue intervening between skin and central axis consists of radiating bundles of areolar and white fibrous connective tissue which anastomose rather freely. Occasional bundles of muscle fibers, somewhat larger than the bundles in specimen A, but much less numerous, may be found in the interstices. The remaining spaces contain blood vessels surrounded by a considerable amount of adipose tissue.

In considering reasons for the state of regeneration found in these specimens, it must first of all be stated that both were victims of a chronic affliction which caused necrosis of bone and muscle. Specimen B had a necrotic mass about the size of a pea in the central axis a centimeter caudal to the point of junction between the original and the regenerated portions of the body. Specimen A showed a necrotic mass in the muscles near the point of fusion. How much importance may be attached to this necrosis is, of course, dubious.

Experimental work thus far done on regeneration of the caudal region of adult reptiles shows the process to be in general as follows: A scab of clotted blood and necrotic material first forms over the wound.[2] This drops in a few days, leaving the stub covered with a layer of unspecialized epithelium which has developed by proliferation of the Malpighian layer of the epidermis. In the regeneration of the central axis, a straight cartilaginous tube is developed which is continuous with the last remaining caudal vertebra. The notochord is not regenerated and no new vertebrae are formed. The cartilaginous tube develops from the periosteum of the uninjured vertebrae or from the bone cells themselves. In the walls of the tube calcareous plates are laid down, which, however, never simulate vertebrae. The neural axis is regenerated in the cavity of the tube from the epithelium of the central canal of the old neural axis. But while it is morphologically a continuation of the old neural axis, it never becomes nervous in structure or function.

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[2] Thomas Hunt Morgan: "Regeneration." The Macmillan Company, 1901; pp. 197 et seq.

Fraisse[3] reaches the following conclusions: "(1). Both in amphibians and in reptiles, injured tissues can only produce new tissues like themselves. The leucocytes assume only the function of nutrition and of devouring the broken-down parts of tissues. They never become fixed tissue . . . . neither connective tissue nor any other sort. (2). All tissues are capable of regenerating themselves, either directly out of their differentiated elements or out of a matrix. As a matrix for the epidermis, there is the Malpighian layer of the skin; for the central nervous system the epithelium of the central canal of the nerve cord; and for the musculature, the spindle fibers."

Barfurth[4] says that two processes may be distinguished in the regeneration of muscle fibers in the mature animal: "(a) degenerative and (b) regenerative. (a) Broken-down muscle fibers that have been cut and torn-off pieces of muscle fibers, are present. There follows an accumulation of leucocytes and of giant cells. The nuclei in the degenerating muscle fibers atrophy and the substance of the fibers breaks down. (b) The muscle fibers split lengthwise to form spindle fibers, and there is an increase in the number of nuclei at the same time. Sarcoblast-like outgrowths of the old muscle fibers are formed, which produce the sarcoblasts that produce new fibers."

Now let us examine the material at hand. Is it possible to correlate the conditions found in the regenerated caudal region of Sphenodon with the known facts of reptilian regeneration? In the opinion of the writer, such a correlation is not at all difficult. The material presents a fairly typical epidermis, a typical central axis, and a typical regenerated substance within that central axis, though the presence of so many large pigment cells may be somewhat anomalous.

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[3] Ibid.: From Fraisse, P. "Die Regeneration von Geweben und Organen bei den Wirbelthieren, besonders bei Amphibien und Reptilien." Kassel und Berlin, 1885.

[4] Morgan: op. cit., p. 200.

The tissues lying between the skin and the central axis are, at first glance, rather extraordinary. It must be remembered that it is impossible to ascertain just what part the accompanying necrosis has played in producing this result. But since the central axis and its contents are normal, the effect of the necrosis in regeneration is probably negligible. The superficial layer of connective tissue may well be considered a product of the proliferation of the cells of the dermis, especially since there is no line of demarcation between the superficial layer and the dermis. The connective tissue of the deeper layer is probably a product of the proliferation of the cells of the fascia surrounding the uninjured muscles. The large amount of adipose tissue in specimen B only indicates a slight modification of the areolar tissue, probably due in part to the accompanying necrosis which would somewhat slow metabolism in the region caudal to the necrotic portion. The tardy regeneration of muscle fibers may be due in part to this same factor. However, muscle fibers are much more numerous in the proximal than in the distal portion of the regenerated region of both specimens, and this would seem to indicate than an insufficient amount of time had elapsed for their complete regeneration.

The specimens studied show, then, atypical but not necessarily abnormal development. The incomplete or abortive regeneration of muscle fibers linked with an overabundance of connective tissue of one sort or another may be explained as due to the accompanying necrosis or to an inherent tardiness of muscle fibers to regenerate. The rest of the regenerated tissues scarcely vary from the type.

PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ANATOMISTS

FORTY-FIRST SESSION

Western Reserve University Medical School, Cleveland, Ohio, April 9, 10, and 11, 1925

Thursday, April 9, 1925

10.00 a.m. The Forty-first Session of the American Association of Anatomists was called to order by President Florence R. Sabin, who appointed the following committees:

Committee on Nominations for 1925: Professor Charles R. Stockard, Chairman, Professor R. R. Bensley, and Professor R. G. Harrison.

Auditing Committee: Professor N. W. Ingalls, Chairman, and Professor A. T. Kerr.

The remainder of the morning session was devoted to the presentation of scientific papers.

Friday, April 10, 1925

12 noon. Association Business Meeting, with President Florence R. Sabin presiding. The Secretary reported that the minutes for the Fortieth Session were printed in full in The Anatomical Record, volume 27, no. 5, pp. 375–382, inclusive. On motion, seconded and carried, the minutes of the Fortieth Session were approved by the Association as printed in The Anatomical Record.

Report of the Auditing Committee: Professor N. W. Ingalls reported for the Auditing Committee as follows: "The undersigned have examined the accounts of the Secretary-Treasurer for the period of December 31, 1923, to December 31, 1924, and have found them to be correct."

[Signed] N. W. Ingalls,
A. T. Kerr.

On motion, the report of the Auditing Committee was accepted and adopted.

Report of the Treasurer: The Treasurer made the following report for the year 1924:

Balance on hand, December 31, 1923 (last audit of accounts)		$194.94	
Receipts from dues (1924)		521.26	
Interest on deposits		5.41	
Total credits			$721.61
Expenditures for 1924:			
Printing and stationery	$105.00		
Postage	40.06		
Expenses of Secretary (Buffalo Meeting)	47.54		
Wistar Institute (for subscriptions)	104.00		
Wistar Institute (for abstracts)	41.70		
Refund (overpayment of dues)	7.00		
Total expenditures			345.30
Balance on hand, December 31, 1924			$376.31

Balance on hand deposited in the name of the American Association of Anatomists in Alexander Brown & Sons, Baltimore, Maryland.

On motion, the report of the Treasurer was accepted.

Election of Officers. Professor W. H. F. Addison, Chairman of the Nominating Committee for 1924, placed before the Association the following nominations for office:

For members of the Executive Committee with term expiring 1928: Professor J. Parsons Schaeffer and Professor T. Wingate Todd.

On motion, seconded and carried, the Secretary was instructed to cast a ballot for the election of the above named.

New Members. The Secretary presented the following names recommended by the Executive Committee for election to membership in the Association:

de Aberle, (Mrs.) Sophie B., A.B., A.M., graduate student, *University of Chicago, Chicago, Ill.*

Adams, A. Elizabeth, A.B., A.M., Ph.D., Associate Professor of Biology, *Mount Holyoke College, Mount Holyoke, Mass.*

Anson, Barry J., A.B., A.M., Fellow in Histology, *Harvard University Medical School, Boston, Mass.*

Butler, Elmer G., A.B., Proctor Fellow in Biology, *Princeton University, Princeton, N. J.*

Chase, Samuel Wood, A.B., A.M., Ph.D., Instructor in Histology and Embryology, *Western Reserve University School of Medicine, 2109 Adelbert Road, Cleveland, Ohio.*

Failey, Gertrude Van Wagenen, A.B., Ph.D., Instructor in Anatomy, *University of California, Berkeley, Calif.*

Faris, Hervey S., A.B., A.M., Ph.D., Instructor in Anatomy, *Loyola University School of Medicine, Chicago, Ill.*

Foster, Wilmot Coyne, A.B., A.M., M.D., Assistant Professor of Anatomy, *University of Oregon Medical School, Portland, Ore.*

Greenhill, J. P., M.D., *426 East Fifty-first St., Chicago, Ill.*

Halpert, Bela, M.D., Instructor in Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*

Holmgren, Nils, M.D., Professor of Zoölogy, *University of Stockholm, Sweden.*

McNutt, G. W., D.V.M., Instructor in Veterinary Anatomy, *Iowa State College, Ames, Iowa.*

Staley, Forest H., A.B., M.D., Instructor in Anatomy, *Washington University, St. Louis, Mo.*

On motion, seconded and carried, all candidates proposed by the Executive Committee were declared duly elected.

Dropped Members. The Secretary then announced the following names as dropped from the list of members on account of non-payment of dues for the past two years:

Dr. C. A. Erdmann, Minneapolis, Minn.
Dr. G. H. Gibson, Otwa, Westland, New Zealand.
Dr. D. M. S. Watson, London, England.

Resignation of Member. The Secretary reported that the following member had resigned from the Association during 1924:

Dr. H. W. Shufeldt, Washington, D. C.

Death of Members. The Secretary then announced, on behalf of the Executive Committee, that during the past year the Association had suffered great loss in the death of the following members:

Dr. Charles Brookover, Louisville, Ky.
Dr. John Irvine Hunter, Sydney, Australia.
Dr. William A. Locy, Evanston, Ill.
Dr. George A. Piersol, Philadelphia, Pa.
Dr. Hubert Sheppard, Emory University, Ga.
Professor John Cleland, London, England, (Honorary member).

Resolution on the death of Professor Piersol: Professor W. H. F. Addison presented to the Association the following resolution regarding the death of Professor George A. Piersol, President of the American Association of Anatomists in 1910–11:

> Dr. George A. Piersol died August 7, 1924, at the age of sixty-eight years, after a long and distressing illness. He was a student of Koelliker and Fritsch during the years 1886–88, after which he returned to the University of Pennsylvania to enter upon his long and uninterrupted service in the Department of Anatomy. His interests were in the presentation of his subject as is shown by his books, including the great human anatomy which he published in 1907. This subject is set forth on the basis of original dissections, skillfully reproduced, and accompanied by a text remarkably lucid and complete. His mind was scholarly, and this trait entered into both his lectures and his writings. As one of the group of editors who founded The American Journal of Anatomy and as member of the original Advisory Board of The Wistar Institute, he was in continuous touch with progress in his chosen field and assisted in the advancement so noticeable during the past twenty years. In 1910 he was president of this Association. In the death of Professor Piersol the Society has lost a member of broad interests and scholarly attainments. By this resolution it wishes to record its appreciation of the life and services of Doctor Piersol and to express its sorrow at his passing.

On motion, duly seconded, it was voted unanimously to spread the above resolution upon the minutes of the Association and the Secretary was instructed to send a copy of the resolution to Mrs. Piersol.

Place of Next Meeting. It was announced that the Executive Committee had decided to hold the next annual meeting at the Sterling Hall of Medicine, Yale University, New Haven, Connecticut, on the days immediately preceding Easter, 1926.

Advisory Editors of Biological Abstracts. The Executive Committee nominated the following members to serve as Advisory Editors in the designated subjects on the Editorial Board of Biological Abstracts, to be published by the Union of American Biological Societies:

Gross Anatomy, Comparative Anatomy, Anthropology: PROF. T. WINGATE TODD.
Neurology: Prof. C. JUDSON HERRICK.
Histology, Embryology: PROF. HERBERT M. EVANS.

On motion, seconded and carried, the Secretary was instructed to cast a ballot for the election of the above named.

Member of Journal Committee. The Executive Committee nominated the following members of the Association, one of whom was to be elected to serve on the Journal Committee as organized at the Thirty-seventh Session of the Association: Professor G. L. Streeter, Professor A. W. Meyer, and Professor H. E. Jordan.

As a result of the election, Professor H. E. Jordan was chosen to serve for five years.

Report of the Journal Committee. Professor G. L. Streeter reported, on behalf of the Journal Committee, that during the past year the anatomical journals had been successfully manufactured in the press of The Wistar Institute. The Association was also informed that no deficit had been incurred by these operations. Reports from the editor of The American Journal of Anatomy and from the editor of The Anatomical Record showed that a large number of manuscripts had been accepted and were awaiting publication.

Report of Committee in Investigate the Status of Anatomy. Professor H. McE. Knower reported, on behalf of the Committee appointed at the Fortieth Session to investigate the status of anatomy, that the Committee had made some progress in its studies, but that no complete investigation of the problem could be made until sufficient funds were obtained. Professor Knower stated that the Committee would continue its efforts to ascertain the exact status of anatomical departments in the various universities in order that problems in teaching and organization could be successfully worked out.

American Association for Medical Progress, Inc. Professor J. M. Rogoff, of Western Reserve University, informed the Association briefly of the aims, purposes, and work of the American Association for Medical Progress, Inc. Professor Davenport Hooker presented the following resolution before the Society:

> WHEREAS: There has been established recently the American Association for Medical Progress, previously known as Friends of Medical Progress—a national lay organization incorporated for the purpose of disseminating medical knowledge among the general public; and
>
> WHEREAS: This American Association for Medical Progress is now actively distributing pamphlets regarding the benefits to humanity derived from animal investigation, vaccination, etc., and in other ways furthering the cause of medical research; be it therefore
>
> *Resolved,* That the American Association of Anatomists, at this, the Forty-first Annual Session, expresses its unqualified approval of the aims, purposes, and work of the American Association for Medical Progress and holds itself willing to cooperate, in any suitable way, with the American Association for Medical Progress.

On motion, duly seconded, the Association unanimously adopted the above resolution.

Dues for the Year 1925. The Executive Committee recommended that the dues for the year 1925 be set at $2.00, of which $1.00 is to be paid to the Union of American Biological Societies for the purpose of aiding in defraying for one year the expenses of the publication of Biological Abstracts.

This recommendation of the Executive Committee was adopted by the Association.

Resolutions proposed for Adoption. Professor Eliot R. Clark presented certain matters of general interest for the consideration of the Association. These resolutions dealt with the following three items:

1. The substitution of work in psychology and sociology for the premedical requirements in biology, as adopted recently by the Association of American Medical Colleges.

2. The desirability of substitution of work in embryology for certain of the premedical requirements in biology.

3. The desirability of the American Association of Anatomists recording itself as opposing the extension of the legislation to prohibit the teaching of evolution.

After discussion, the Association voted to refer these matters to the Executive Committee for report to the Association at the concluding business session.

Arrangement of Program. Professor Charles R. Bardeen proposed that the sessions of the Association devoted to the reading of papers be so arranged that free discussion of the papers can be held. Doctor Bardeen's proposal suggested that no papers as such be given, but that the time allotted to each speaker be devoted largely to the demonstration of slides and other scientific exhibits.

On motion, Doctor Bardeen's proposal was referred to the Executive Committee.

Traveling Expenses to Meetings. Professor R. Bennet Bean spoke briefly regarding the desirability of having universities defray the expenses incurred by members of the faculties in attendance at scientific meetings. No action was taken.

On motion, the business session then adjourned.

SATURDAY, APRIL 11, 1925

12.45 p.m. A short business meeting followed the morning scientific session, which concluded with the address of the President on the "Opportunity of Anatomy."

The Secretary reported that the Executive Committee had decided to refer the proposals of Professor Clark, regarding the substitution of psychology, sociology, and embryology for the premedical requirements in biology, to the Committee to Investigate the Status of Anatomy. The Executive Committee further suggested that it would be unwise to take any official action regarding the antievolution movement at this time, as it had been informed that a lay committee was in process of formation for the purpose of opposing any extension of the legislation to prohibit the teaching of evolution. The Executive Committee felt that when this lay society should become fully organized endorsement of its work should be given by the American Association of Anatomists.

No objection to these decisions of the Executive Committee was expressed.

Professor C. M. Jackson presented the following resolution:

Resolved: That we express our hearty appreciation of the hospitality extended to us by the Western Reserve University on this occasion. We are especially indebted to Professors Todd and Waite and their associates for the facilities and arrangements which have contributed so much to the success of the present meeting.

Moved, seconded, and unanimously voted.

On motion, the Session adjourned.

LEWIS H. WEED,
Secretary of the Forty-first Session of
the American Association of Anatomists.

AMERICAN ASSOCIATION OF ANATOMISTS

OFFICERS AND LIST OF MEMBERS

Officers

President	Florence R. Sabin
Vice-President	Arthur W. Meyer
Secretary-Treasurer	Lewis H. Weed

Executive Committee

For term expiring 1925	Davenport Hooker, Benjamin F. Kingsbury
For term expiring 1926	H. McE. Knower, Richard E. Scammon
For term expiring 1927	Charles R. Stockard, Clarence M. Jackson
For term expiring 1928	J. Parsons Schaeffer, T. Wingate Todd

Members of Journal Committee and Expiration of Term

H. M. Evans (1926)
Frederic T. Lewis (1927)
Charles R. Bardeen (1928)
Clarence M. Jackson (1929)
Harvey E. Jordan (1930)

Delegate to the Council of A. A. A. S.

Harold D. Senior

Representative to the National Research Council

H. H. Donaldson

Committee on Nominations for 1925

Charles R. Stockard, *Chairman*
R. R. Bensley
R. G. Harrison

HONORARY MEMBERS

S. Ramón y Cajal	*Madrid, Spain*
Camille Golgi	*Pavia, Italy*
A. Nicolas	*Paris, France*
Wilhelm Roux	*Halle, Germany*

Members

Abbott, Maude E., A.B., C.M., M.D., F.R.C.P.& S. Professor of Pathology, Women's Medical College, *1300 Spruce St., Philadelphia, Pa.*

de Aberle, (Mrs.) Sophie B., A.B., A.M., Graduate student, *University of Chicago, Chicago, Ill.*

Adams, A. Elizabeth, A.B., A.M., Ph.D., Associate Professor of Biology, *Mount Holyoke College, Mount Holyoke, Mass.*

Adams, L. A., A.B., A.M., Ph.D., Assistant Professor of Zoölogy, *University of Illinois, 605 West Indiana St., Urbana, Ill.*

Addison, William Henry Fitzgerald, B.A., M.D., Professor of Normal Histology and Embryology, *School of Medicine, University of Pennsylvania, Philadelphia, Pa.*

Adelmann, Howard B., B.A., M.A., Ph.D., Instructor in Histology and Embryology, *Cornell University, Stimson Hall, Ithaca, N. Y.*

Alford, Leland Barton, A.B., M.D., Associate in Clinical Neurology, Washington University School of Medicine, *Humboldt Building, St. Louis, Mo.*

Allen, Bennet Mills, Ph.D., Associate Professor of Biology, University of California (Southern Branch), *909 Micheltorena St., Los Angeles, Calif.*

Allen, Edgar, A.M., Ph.D., Professor of Anatomy, *University of Missouri, Columbia, Mo.*

Allen, Ezra, A.M., Ph.D., Professor of Biology, *Ursinus College, Collegeville, Pa.*

Allen, William F., A.M., Ph.D., Professor of Anatomy, *University of Oregon Medical School, Portland, Oregon.*

Allis, Edward Phelps, Jr., C.E., M.D., LL.D., Palais de Carnoles, *Menton (A.M.)*, France.

Anson, Barry J., A.B., A.M., Fellow in Histology, *Harvard University Medical School, Boston, Mass.*

Appleby, J. I., A.B., M.B., A.M., M.D., *105½ West Main St., Bellevue, Ohio.*

Arai, Hayato, M.D., Chief Gynecologist, *Sapporo Hospital, Sapporo, Japan.*

Arey, Leslie B., Ph.D., Professor of Microscopic Anatomy, Northwestern University Medical School, *2421 Dearborn St., Chicago, Ill.*

Atterbury, Ruth Rand, A.M., Ph.D., *565 Park Ave., New York City.*

Atwell, Wayne Jason, A.M., Ph.D., Professor of Anatomy, University of Buffalo Medical College, *24 High St., Buffalo, N. Y.*

Badertscher, Jacob A., Ph.D., Professor of Anatomy, Indiana University School of Medicine, *312 South Fess Ave., Bloomington, Ind.*

Bagley, Charles, Jr., M.D., *The Latrobe Apts., Baltimore, Md.*

Bailey, Percival, M.D., Ph.D., Junior Associate in Surgery, Peter Bent Brigham Hospital, *721 Huntington Ave., Boston, Mass.*

Baitsell, George Alfred, M.A., Ph.D., Assistant Professor of Biology, Yale University, *Osborn Zoölogical Laboratory, New Haven, Conn.*

Baldwin, Wesley Manning, A.B., A.M., M.D., Professor of Anatomy, *Union University Medical College, Eagle St., Albany, N. Y.*

Bardeen, Charles Russell, A.B., M.D. (Ex. Com. '06–'09, Vice-President '18–'20), Professor of Anatomy and Dean of Medical School, *University of Wisconsin, Science Hall, Madison, Wis.*

Barta, Edmund, M.D., Adjunctus at the University of Budapest, Fellow Rockefeller Foundation, *Washington University, St. Louis, Mo.*

Bartelmez, George W., Ph.D., Associate Professor of Anatomy, *University of Chicago, Chicago, Ill.*

BARTSCH, PAUL, A.B., M.S., Ph.D., Curator, Division of Mollusks, *U. S. National Museum, Washington, D. C.*

BASCOM, KELLOGG F., Ph.D., Instructor in Histology and Embryology, *University of Pennsylvania, Philadelphia, Pa.*

BAST, T. H., B.A., Ph.D., Assistant Professor of Anatomy, *University of Wisconsin, 308 Science Hall, Madison, Wis.*

BATES, GEORGE ANDREW, M.S., D.M.D., Professor of Histology and Embryology, Tufts College Medical School, *305 Central Ave., Auburndale, Mass.*

BATSON, O. V., A.M., M.D., Associate Professor of Anatomy, *College of Medicine, Cincinnati University, Eden Ave., Cincinnati, Ohio.*

BAUMGARTNER, WILLIAM J., A.B., A.M., Associate Professor of Zoölogy, University of Kansas, *1209 Ohio St., Lawrence, Kans.*

BAYON, HENRY, B.A., M.D., Professor of Applied Anatomy, Tulane University, *2212 Napoleon Ave., New Orleans, La.*

BEAN, ROBERT BENNETT, B.S., M.D., Professor of Anatomy, University of Virginia, *5 Wayside Place, University, Va.*

BEGG, ALEXANDER S., B.S., M.D., Professor of Anatomy, Boston University School of Medicine, *80 East Concord St., Boston, Mass.*

BENSLEY, ROBERT RUSSELL, A.B., M.B., Sc.D. (Second Vice-Pres. '06–'07, Ex. Com. '08–'12, President '18–'20), Professor of Anatomy, Director of Hull Laboratory of Anatomy, *University of Chicago, Chicago, Ill.*

BEVAN, ARTHUR DEAN, M.D. (Ex. Com. '96–'98), Professor of Surgery, University of Chicago, *122 South Michigan Blvd., Chicago, Ill.*

BIGELOW, ROBERT P., S.B., Ph.D., Associate Professor of Zoölogy and Parasitology, *Massachusetts Institute of Technology, Cambridge 39, Mass.*

BISHOP, KATHERINE SCOTT, A.B., M.D., Histologist, Hooper Research Institute, San Francisco, *1508 La Loma Ave., Berkeley, Calif.*

BLACK, DAVIDSON, B.A., M.B., Professor of Anatomy, *Peking Union Medical College, Peking, China.*

BLAIR, VILRAY PAPIN, A.M., M.D., Associate in Clinical Surgery, Washington University School of Medicine, *Metropolitan Building, St. Louis, Mo.*

BLAISDELL, FRANK ELLSWORTH, SR., M.D., Associate Professor of Surgery, Medical Department of Stanford University, *1520 Lake St., San Francisco, Calif.*

BLAKE, J. A., A.B., Ph.B., M.A., M.D., *116 East Fifty-third St., New York City.*

BOYDEN, EDWARD ALLEN, A.M., Ph.D., Assistant Professor of Comparative Anatomy, *Harvard Medical School, 61 Clarls St., Newton Centre, Mass.*

BREMER, JOHN LEWIS, A.B., M.D. (Ex. Com. '15–'18), Associate Professor of Histology, *Harvard Medical School, Boston, Mass.*

BROADNAX, JOHN W., Ph.G., M.D., Associate Professor of Anatomy, Medical College of Virginia, *1429 Bainbridge St., Richmond, Va.*

BROWNING, WILLIAM, Ph.B., M.D., Professor of Neurology, Long Island College Medical School, *54 Lefferts Place, Brooklyn, N. Y.*

BRYCE, THOMAS H., M.A., M.D., F.R.S., Professor of Anatomy, University of Glasgow, *No. 2, The University, Glasgow, Scotland.*

BULLARD, H. HAYS, A.M., Ph.D., M.D., Professor of Pathology, *Western University Medical School, London, Ontario, Canada.*

BUNTING, CHARLES HENRY, B.S., M.D., Professor of Pathology, *University of Wisconsin, Madison, Wis.*

Burr, Harold Saxton, Ph.D., Assistant Professor of Anatomy, School of Medicine, Yale University, *333 Cedar St., New Haven, Conn.*

Burrows, Montrose T., A.B., M.D., Associate Professor of Experimental Surgery, Director of Research Laboratories, Barnard Free Skin and Cancer Hospital, *Washington University Medical School, St. Louis, Mo.*

Butler, Elmer G., A.B., Proctor Fellow in Biology, *Princeton University, Princeton, N. J.*

Calkins, Leroy A., B.S., M.S., M.B., M.D., Ph.D., *Box 306, University, Va.*

Cameron, Angus L., A.B., M.S., M.D., Ph.D., Assistant Professor of Surgery, *Medical School, University of Minnesota, Minneapolis, Minn.*

Cameron, John, M.D., D.Sc., F.R.S.E., Professor of Anatomy, *Dalhousie Medical College, Halifax, Nova Scotia.*

Campbell, William Francis, A.B., M.D., Professor of Anatomy and Histology, Long Island College Hospital, *1835 East New York Ave., Brooklyn, N. Y.*

Cardwell, John C., M.D., Professor of Physiology, Long Island College Hospital, *Polhemus Memorial Clinic, Brooklyn, N. Y.*

Carey, Eben J., M.S., Sc.D., Dean of Students, Professor of Anatomy, Marquette University School of Medicine, *638 Fourth St., Milwaukee, Wis.*

Carpenter, Frederick Walton, Ph.D., Professor of Biology, *Trinity College, Hartford, Conn.*

Carter, James Thornton, D.D.S., Research Assistant, Department of Zoölogy, Assistant in Embryology and Histology, *University College, 1 Hanover Square, London, W. C. 1, England.*

Carver, Gail L., A.B., A.M., *West Lake, Ga.*

Casamajor, Louis, A.M., M.D., Professor of Neurology, Columbia University, *437 West 59th St., New York City.*

Chagas, Carlos P., M.D., Professor of Histology and Pathology, *Bello Horizonte Medical School, Minas-Geraes, Rua Tymbiras 1490, Brazil, South America.*

Chambers, Robert, Jr., A.M., Ph.D., Professor of Microscopic Anatomy, *Cornell University Medical College, New York City.*

Chang, Chun, M.D., Professor of Anatomy, *Hopei University, Paotingfu, Chihli, China.*

Charlton, Harry Hayward, A.B., A.M., Ph.D., Associate Professor of Anatomy, *Department of Anatomy, University of Missouri, Columbia, Mo.*

Chase, Samuel Wood, A.B., A.M., Ph.D., Instructor in Histology and Embryology, *Western Reserve University School of Medicine, 2109 Adelbert Road, Cleveland, Ohio.*

Chidester, Floyd E., A.M., Ph.D., Professor of Zoölogy, *University of West Virginia, Morgantown, W. Va.*

Child, Charles Manning, M.S., Ph.D., Professor of Zoölogy, *Zoölogical Laboratory, University of Chicago, Chicago, Ill.*

Chillingworth, Felix P., M.D., Professor of Physiology and Experimental Pharmacology, Tufts Medical College, *416 Huntington Ave., Boston, Mass.*

Chouke, Kehar Singh, M.D., *4200 East Ninth Ave., Colorado University Medical School, Denver, Colo.*

Clark, Eleanor Linton, A.M., Research Worker, Department of Anatomy, *Medical Department, University of Georgia, Augusta, Ga.*

Clark, Eliot R., A.B., M.D. (Ex. Com. '16–'19), Professor of Anatomy, *Medical Department, University of Georgia, Augusta, Ga.*

Coe, Wesley R., Ph.D., Professor of Biology, *Yale University, Osborn Zoölogical Laboratory, New Haven, Conn.*

Coghill, George E., A.B., M.S., Ph.D., Professor of Anatomy and Head of Department, University of Kansas Medical School, Department of Anatomy, University of Kansas, *R.F.D. 9, Lawrence, Kan.*

Cohn, Alfred E., A.B., M.D., Member, Rockefeller Institute for Medical Research, *New York City, N. Y.*

Conant, William Merritt, A.B., M.D., Professor of Clinical Surgery, *486 Commonwealth Ave., Boston, Mass.*

Conel, Jesse LeRoy, A.M., Ph.D., Associate Professor of Anatomy, Boston University School of Medicine, *80 E. Concord St., Boston, Mass.*

Congdon, Edgar Davidson, Ph.D., Associate Professor of Anatomy, *Peking Union Medical College, 26 S. Compound, Peking, China.*

Conklin, Edwin Grant, A.M., Ph.D., Sc.D., Professor of Biology, Princeton University, *139 Broadmead Ave., Princeton, N. J.*

Corner, George W., A.B., M.D., Professor of Anatomy, *University of Rochester, Rochester, N. Y.*

Corning, H. K., M.D., Professor of Anatomy, *University of Bâle, Anatomische Anstalt, Bâle, Switzerland.*

Cowdry, Edmund V., Ph.D., Associate Member, *Rockefeller Institute for Medical Research, Ave. A. and 66th St., New York City.*

Craig, Joseph David, A.M., M.D., *12 Ten Broeck St., Albany, N. Y.*

Craigie, E. Horne, B.A., Ph.D., Lecturer in Comparative Anatomy and Neurology, *Department of Biology, University of Toronto, Toronto, Canada.*

Crile, George W., A.M., M.D., LL.D., F.A.C.S., Professor of Surgery, Western Reserve University, *Cleveland Clinic, 93rd and Euclid, Cleveland, Ohio.*

Crosby, Elizabeth Caroline, M.S., Ph.D., Instructor in Anatomy, University of Michigan, *420 Maynard St., Ann Arbor, Mich.*

Cullen, Thomas S., M.B., Professor of Clinical Gynecology, Johns Hopkins University, *20 E. Eager St., Baltimore, Md.*

Cummins, Harold, A.B., Assistant Professor of Anatomy, *Tulane University Medical School, Department of Anatomy, Sta. 20, New Orleans, La.*

Cunningham, Robert S., A.M., M.D., Associate Professor of Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*

Curtis, George M., A.M., Ph.D., M.D., Medical Fellow, National Research Council, Research Fellow in Anatomy, University of Chicago, *1753 W. Congress St., Chicago, Ill.*

Dahlgren, Ulric, A.B., M.S., Professor of Biology, Princeton University, *204 Guyot Hall, Princeton, N. J.*

Danchakoff, Vera, M.D., Assistant Professor of Anatomy, College of Physicians and Surgeons, Columbia University, *437 W. 59th St., New York City.*

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CONTRIBUTIONS FROM THE DEPARTMENT OF ANATOMY, Peking Union Medical College, Peking, China, vol. 2, 1921–22, Collected papers nos. 17–38, by N. H. Cowdry, S. R. Detwiler, E. V. Cowdry, P. H. Stevenson, Henry Laurens, Davidson Black: Reprints from *Biological Bulletin, Proceedings of the National Academy of Sciences, Annals of Medical History, The China Medical Journal, The Anatomical Record, The Journal of Comparative Neurology,* and *The Journal of Experimental Zoölogy.*

THE MICROSCOPE, An Introduction to Microscopic Methods and to Histology, Dark-field Edition (14th) Revised, by Simon Henry Gage, Professor of Histology and Embryology, Emeritus, in Cornell University, 1925; 518 pages, 272 figures. Size 6 × 9, cloth. The Comstock Publishing Company, Ithaca, N. Y. ''In revising and rewriting this book now for the fourteenth time, the aim has been as in all previous editions, to give the student the benefit of the fundamental things which have been worked out in microscopy. The opportunities given by the freedom from teaching have rendered it possible to make this revision more thorough than could be done in any previous edition. Progress in all that pertains to microscopy has been marked during the past ten years. Nothing fundamentally new has appeared.''

THE EARLY EMBRYOLOGY OF THE CHICK, by Bradley M. Patten, Associate Professor of Histology and Embryology, School of Medicine, Western Reserve University. Second Edition, 1925, 178 pages, 64 illustrations (213 figures, frontispiece in colors), Appendix and Index, 6 × 9¼, cloth. P. Blakiston's Son & Co., Philadelphia. *From the Preface*: ''The primary purpose of writing this book was to provide a brief and simple text for the beginning student. In making a revision I have attempted to improve the presentation—rather than to expand it.''

THE ANATOMY OF THE NERVOUS SYSTEM, by Stephen W. Ransom, M.D., Ph.D., Professor of Anatomy in Northwestern Medical School, Chicago. Second Edition, 1925, 422 pages, 284 figures, Bibliography, Index. Size 6¾ × 10¼, cloth, $6.50; W. B. Saunders Company, Philadelphia, Pa. ''There are very definite limits to the amount of detail the novice in neurology can be expected to master. Additions to the text of the first edition have, therefore, been restricted to a minimum. Space has been found for the really important advances. The more important additions are: a brief account of the blood supply of the brain, a series of figures representing sections through the basal ganglia and internal capsule, and brief accounts of twelve clinical cases selected to illustrate the functional significance of the various parts of the nervous system.''

DYNAMIC PSYCHOLOGY, An Introduction to Modern Psychological Theory and Practice, by Dom Thomas Verner Moore, Ph.D., M.D., Monk of the Order of St. Benedict, Professor of Psychology, Catholic University of America, Director of the Clinic for Mental and Nervous Diseases, Providence Hospital, Washington, D. C., 1924, 444 pages, 6 figures, Glossary and Subject Index, 5½ × 8¼, cloth,

J. B. Lippincott Company, Philadelphia. The book is divided into six parts: Part I, The analysis of mind; Part II, Stimulus and response and human behavior; Part III, Human emotional life; Part IV, The driving forces of human nature and their adjustment; Part V, Psychoanalysis and psychotherapy; Part VI, Volitional control.

BRITISH HYMENOPTERA, by A. S. Buckhurst, A.R.C.S., D.I.C., L. N. Staniland, A.R.C.S., D.I.C., and E. B. Watson, A.R.C.S., with an Introduction by H. M. Lefroy, M.A., 1923, 56 pages, 62 figures, size 7½ × 9¾, cloth, $3.00, London, England: Arnold & Co.; New York: Longmans, Green & Co. ''This little book is intended to be a help to those who desire to commence the study of a large and much-neglected group of insects, bees, wasps, and ants, of which there are some four thousand species.'' There is a brief Bibliography and an Index.

RUBBER AND GUTTA PERCHA INJECTIONS, Subcutaneous injections of rubber and gutta percha for raising the depressed nasal bridge and altering external contours, by Charles Conrad Miller, M.D., 1923, 100 pages, 10 figures, Size 5¼ × 7¾, cloth, $1.75. Oak Printing and Publishing Company, Chicago, Illinois. Explanation: ''It may appear strange to some readers that products so simple as crude rubber and gutta percha should not only be described in the text but illustrated as well.

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QUAIN'S ELEMENTS OF ANATOMY, Eleventh Edition, in four volumes—Volume 4, Part 2, *Myology*, by T. H. Bryce. Sections on the Actions of Muscles by Thomas Walmsley, M.D., 1923. 310 pages, 53 colored plates and other illustrations, size 6¼ × 9¾, cloth, $8.00. Longmans, Green & Co., New York, London, Toronto, Calcutta. ''Much attention had been directed to the muscles as a result of the surgical experience of the war. It was therefore determined to make elaborate the portion on the muscles into a special volume dealing in detail with the muscular system as a whole'' and ''to summarize the large amount of work which has been done in the last twenty-five years on the morphology of the muscles.''

INTERNATIONAL CLINICS, Volume 3, Thirty-third Series. A Quarterly of Illustrated Clinical Lectures and especially prepared original articles by twenty-eight authors, fully illustrated; 1923, 312 pages, size 6 × 9, cloth. J. B. Lippincott Company, Philadelphia and London.

PAPERS BY AMERICAN CONTRIBUTORS to the Second International Congress of Military Medicine and Pharmacy, Rome, May–June, 1923. Reprinted from *The Military Surgeon*, Washington, D. C., 1924. Five papers, size 7 × 10, paper cover, 120 pages, illustrated by charts. Contents. The Influence of the World War on Surgical Tuberculosis, America's Viewpoint, Wm. S. Bainbridge; The Utility of Serums and Vaccines in War, Geo. W. McCoy; Military Mental Defects, R. F. Sheehan; Tuberculosis as a War Problem, Philip R. Jacobs, Ph.D.; The Experience of the U. S. Navy in the Prevention and Control of Venereal Disease during and since the World War, Robert F. Jones.

ACCESSORY PARATHYROIDS IN THE RAT

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ONE FIGURE

At the meeting of the American Association of Anatomists in 1924 the authors of this paper presented the results of a series of parathyroidectomy operations which they had performed on rats. The discussion of that report all turned on the question whether the operations were in fact complete parathyroidectomies, and whether it is possible to remove all the parathyroid tissue from a rat. We felt it advisable to consider this aspect of the problem before going any further in our study of the conditions affecting the susceptibility of rats to tetania parathyreopriva; and the results of our study are presented herewith.

It has been stated by some writers that accessory parathyroids are common in rats. If they occur frequently, results of operations in which the two normally placed glands are removed lose their value. Erdheim ('06) made serial sections of all the organs of the neck, including the thymus. His series included ten rats, three of which were wild gray and the rest albinos. He states that accessory parathyroids were found in every case. The most common location for them was in the thymus embedded in the parenchyma or in the connective-tissue stroma. Less frequently he found them in the connective tissue between the thyroid and the thymus, accompanied by masses of thymic tissue.

More recently, the subject of accessory parathyroids has been discussed by Shapiro and Jaffe ('23). They quote a paper by Togofuku in which the number of accessory para-

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thyroids in one rat is said to have been eleven. They state that there is at present no evidence that mammals can survive the operation of parathyroidectomy if it be complete.

The material used by us for this study is of several sorts. First, we have cut serial sections of rat embryos, to include all the organs and connective tissue between the lower jaw and the heart. The embryos were from white and gray mothers and were old enough so that the parathyroids in their normal situation were clearly recognizable. We have also studied serial sections of the neck regions from adult rats. In these series are included the trachea, esophagus, and thyroid, and the immediately adjacent muscles and connective tissue. The trachea and esophagus were cut at the level of the pharynx and again at the level of the clavicle. This material was almost all from operated animals, many of which had lived for some time after the operation. It would be reasonable to expect that any accessory parathyroids in these animals would have undergone hypertrophy following the operation and be readily detectable. Thirdly, we have examined complete serial sections of a number of thymi; and finally serial sections were made from young rats, in which the trachea, esophagus, glands, and muscles were dissected out together with the fascia from the pharynx through the thymus.

A table of this material is presented below (table 1).

The position of the accessory parathyroid in the five specimens in which it was found is worth noting. We have never found a specimen with more than one accessory, and in four of the five cases this one lay in the position shown in the accompanying figure: cephalad to the thyroid and dorsal to the cartilage of the larynx. The fifth specimen had a small parathyroid deeply embedded in the thyroid, and in this case one of the two parathyroids removed by the operation was far below the normal size. The piece of gland removed had a complete connective-tissue capsule. Evidently, the parathyroid on this side was separated into two small masses, both of which were in the normal position of the gland.

It seems to us, after a study of these sections, that the rat furnishes very favorable material for the study of the results of parathyroidectomy. It has already been shown by Zuckerkandl that there are but two parathyroid anlagen; and we are convinced that accessories are so infrequent as to be negligible in properly controlled experiments. Conditions are evidently different from those in the cat and dog where a large percentage of cases show aberrant masses of parathyroid tissue. We have observed that in these animals the thymic tissue is also somewhat scattered. Almost every specimen of cat thyroid includes some thymic tissue. In the rat the thymus is more compact. While masses of thymic tissue are sometimes found connected with the thyroid of the rat, their occurrence is not nearly so frequent as in the cat.

TABLE 1

	NO ACCESSORY PARATHYROID	ACCESSORY PARATHYROID
Embryos:		
A. Series including all structures from level of lower jaw to heart	14	
B. Series including all structures from level of thyroid to heart	16	
Newborn to thirty days:		
A. Series including all structure from level of lower jaw to heart	6	
B. Series including trachea, esophagus, thyroid, and thymus, with surrounding fascia	4	
Adult rats:		
A. Series including trachea and esophagus with surrounding muscles and fascia	39	5
B. Thymus only	15	

We feel sure that in a long series of experiments with rats one could count on having only a small proportion of the results vitiated by the presence of accessory parathyroids. Moreover, it is reasonable to suppose that the percentage of accessories would be the same in any two large groups, so that in experiments comparing the effect of parathyroidectomies under different conditions the question of accessories may be disregarded. Finally, since we have never found accessories outside the region of the pharynx and trachea, we consider that sections of the neck region give a satisfactory check of the completeness of the operation in individual cases.

We cannot explain the discrepancy between these results and those of Erdheim and Togofuku. Were it a matter of slight differences in numbers, we might believe our technique or observation to be at fault; but we cannot believe that we should fail to see accessory parathyroids in so many cases if they were present in all rats. It is of course possible that conditions vary in such widely different localities. In that case, our results are valid only for our own groups of rats.

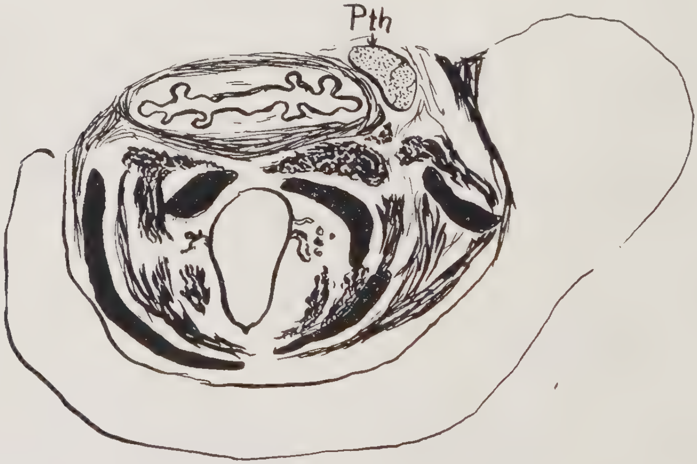

Figure 1

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THE BLOOD OF NORMAL MICE

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ONE FIGURE

In the course of an investigation of leukemias and related conditions in the Slye stock of mice, it became necessary to make a careful study of the blood of normal mice. The meagerness of the available literature on this subject and the fact that mice are now frequently used in the study of the effects of various physical and chemical agents on the blood-forming organs appeared to justify the publication of these results.

Goodall[1] found that the erythrocytes of normal mice varied in number from 6,000,000 to 10,000,000 per cu.mm. and that the leucocytes ranged from 8000 to 10,000 per cu.mm. Young[2] gives the normal leucocyte count of mice as 5000 per cu.mm. The percentages of the different types of leucocytes recorded by these authors were as follows:

	Goodall	*Young*
	Per cent	Per cent
Lymphocytes	71.00	62.44
Polymorphonuclears	23.00	36.40
Eosinophiles	5.75	0.69
Basophiles	0.25	0.10

There is a marked difference of opinion as to the presence of granules in the leucocytes of mice. Stäubli[3] described briefly two varieties of leucocytes in the blood of mice: mononuclears, which often have vacuoles in the peripheral portion of their cytoplasm, and polymorphonuclears. He found it difficult to demonstrate granules in the latter, a considerable proportion of these cells showing no granules whatever. He differentiated two subclasses of polymorphonuclear leucocytes: *a*) those with finely granular cytoplasm and nuclei composed of from four to six lobes or in the form of a ring and, *b*) polymorphonuclears with coarsely granular cytoplasm which stained with eosin. Stäubli does not mention the relative numbers of these types of leucocytes in the blood.

In an illustration in his article on an "Experimental Study of Metastases" (in mice) Takahashi[4] shows lymphocytes and large cells with definitely granular cytoplasm and nuclei that are polymorphic in form.

Furno[5] studied the leucocytes of white mice and reported that the distinctness of their granules varied with the stain used and with the length of time it was allowed to act. He was able to demonstrate granules in the cytoplasm of the polymorphonuclear leucocytes. Schifone[6] and Mezinescu[7] described neutrophilic granules in the leucocytes of white mice. Mueller[8] observed granules in polymorphonuclear leucocytes by dark-ground illumination. This was confirmed by Hirschfeld[9], who denies the possibility of staining them. Bunting[10] has recently stated that the polymorphonuclears of mice do not possess granules.

Hirschfelder,[11] on the other hand, used triacid and basic stains and differentiated four types of cells in mouse blood: *a*) polymorphonuclear eosinophiles with granules about the size of those in human neutrophilic leucocytes; *b*) polymorphonuclear leucocytes without granules and with quite homogeneous cytoplasm; *c*) lymphocytes, and, *d*) mast cells. The polymorphonuclear leucocytes frequently had ring-shaped nuclei.

Murphy and Sturm[12] obtained much higher leucocyte counts in their normal mice than any others recorded. Their figures range from 10,800 to 24,000 per cu.mm.; the lymphocytes varied from 54 to 65 per cent and the polymorphonuclears from 35 to 46 per cent.

My own study of the blood cells of mice has included an examination of the cells of the blood itself and direct smears from the marrow of the upper end of the femur, from the sternum and the bodies of the vertebrae. These smears were stained by Wright's stain for routine study.

In our series the number of erythrocytes in the blood of normal mice ranged from 6,000,000 to 8,000,000 per cu.mm. The mouse red cells are biconcave discs without nuclei, slightly smaller than the corresponding cells of man, and quite uniform in size and shape. No nucleated red cells were found in any of the smears examined. About 10 per cent of the erythrocytes showed a noticeable polychromatophilia. No basophilic degeneration was observed.

The nucleated cells from the blood and bone-marrow include lymphocytes, monocytes (transitionals), and polymorphonuclears, and can be discussed together. The total leucocyte counts in this series of mice ranged between 6000 and 11,000 per cu.mm. In smears from the bone-marrow, in addition to the nucleated cells encountered in the blood, there were numerous large mononuclear cells with faintly granular cytoplasm and resembling myelocytes.

The lymphocytes of normal mouse blood resemble closely those found in the blood of man and other mammals. They are slightly larger than erythrocytes and have a round or oval nucleus that almost completely fills the cell. The relative proportion of the cells in the blood varied in different mice, but was uniformly higher than in man. The number rarely fell below 50 per cent and rarely exceeded 60 per cent of the total leucocyte count.

The large mononuclears or monocytes have large eccentrically placed nuclei that may be round, oval, kidney-shaped, or horseshoe-shaped. Their cytoplasm is relatively abundant and usually contains vacuoles situated at the periphery of the cell. The proportion of these cells ranged from 3 to 10 per cent of the total number of leucocytes.

Polymorphonuclear leucocytes of mice have the chief characteristics of the corresponding cells in human blood. Their nuclei are variously shaped; their cytoplasm may be entirely free from granules, but granules can usually be rendered visible by prolonged staining with Wright's stain. They do not take the dye readily nor as deeply as the granules in the leucocytes in the blood of man. The relative numbers of these cells vary from 24 to 50 per cent of the total leucocyte count. Eosinophiles were present in the proportion of 0.5 to 3 per cent. Very few basophiles were encountered; they constituted less than 1 per cent of the total number of leucocytes. The differential counts in this series of mice may be summarized as follows:

	Per cent
Lymphocytes,	50 to 65
Polymorphonuclears,	24 to 50
Eosinophiles,	0.5 to 3
Large mononuclears,	3 to 10
Basophiles,	less than 1

The polymorphonuclear leucocytes can be advantageously classified on the basis of the shape of the nucleus. After a study of the cells of the bone-marrow, it appears that this method of classification gives an indication of the stage of maturity of a given polymorphonuclear leucocyte. The nuclei have the following shapes: Complete ring; broken ring; segmenting ring; knot or loop; multilobular, or definitely polymorphonuclear. In any blood smear numerous nuclei are seen that combine the characteristics of two of these types.

The complete ring nucleus is composed of a ring-shaped mass of nuclear material that suggests a doughnut in form and general outline. This appears to be the basis from which the more irregular forms of nuclei develop. In the bone-marrow it is possible to trace the development of these nuclei from the large round nuclei of the myelocytes mentioned above. One finds in the marrow, large cells in all essential respects like these myelocytes except that the nucleus shows a tiny opening at its center. Along with these are other cells with openings in their nuclei of varying size up to relatively large ones. But the nuclear material always forms a complete unbroken ring. It is apparently to this type of cell that Tyzzer[13] refers when he mentions the 'large hollow nuclei.' He states that "large numbers of cells of the myelocyte type are found in certain areas, and they are often intermingled with the tumor tissue just described. They are distinguished from the cells of the latter by their smaller size, by the basophilic property of their cytoplasm, by the frequent ring-shaped nucleus, and in some cases by the demonstrable granules'' (p. 494). Tyzzer, therefore, classes these cells as myelocytes. Fajersztajn and Kuczynski[14] observed great numbers of cells of this type in the bone-marrow of mice.

Inasmuch as these cells are found quite regularly, even though in small numbers, in the circulating blood of normal mice, it does not appear to be proper to designate them as myelocytes. It would seem to be more correct to class them as early or very young forms of polymorphonuclears. They are enormously increased in number in myelogenous leukemia in mice and take a prominent place in the foci of myeloid infiltration of the viscera so characteristic of this disease.

This unbroken nuclear ring appears to form the basis for further changes in the shape of the nucleus as the process of maturing proceeds. The ring of nuclear material may break in one place and the ends become separated, either in the same or different planes. In such a nucleus the caliber of the cylindrical mass is rather uniform throughout. Instead of breaking in one place, the ring may begin to form constrictions at several places in its circumference and the process of segmentation begins. There is thus formed a roughly ring-shaped nucleus made up of irregular masses of nuclear material joined together by narrow bands of the same material.

Without breaking in one place or becoming segmented the ring of nuclear matter appears to become smaller in caliber and at the same time of greater circumference, so that it becomes coiled up within the cell in the form of fantastic knots or loops. These loops or knots appear later to break in one or several places, thus forming broken or segmenting knots or loops.

In a fairly small percentage of these cells the fragments of the segmenting rings or loops become so displaced and separated that the ring or loop or knot form is no longer distinguishable. These have been classed as multilobular or definitely polymorphonuclear, and appear to be the oldest and most mature type of polymorphonuclear leucocyte.

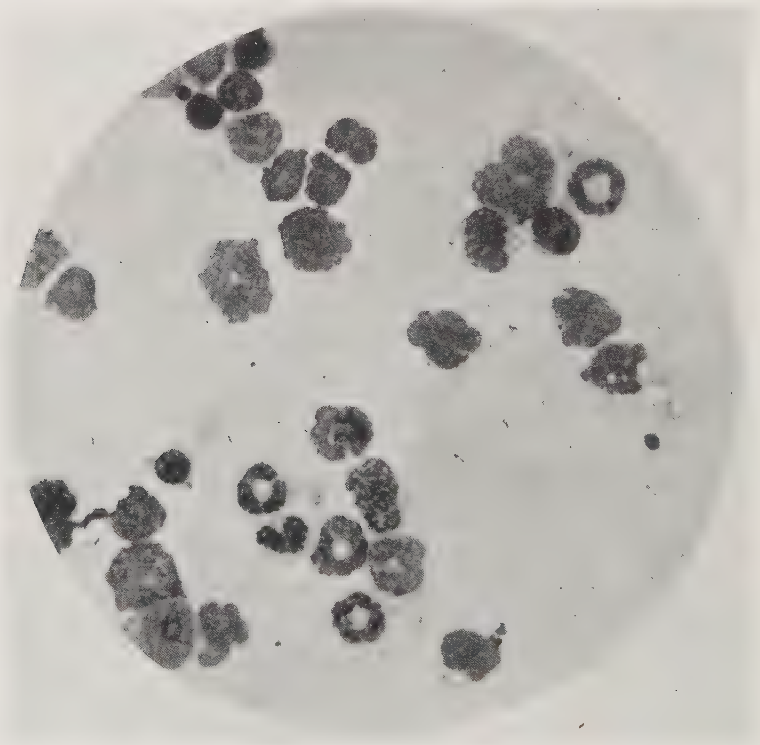

Fig. 1 Smear from bone-marrow of the vertebra of a mouse. × 750. The cells with large solid nuclei are myelocytes. The stages in the formation of the ring- or doughnut-shaped nuclei are well shown.

The percentages of these five different types of leucocytes vary somewhat in different mice. It can be stated as a general rule, however, that nuclei of the unbroken-ring type are the least numerous in the circulating blood, and that those having knot- or loop-like nuclei are the most abundant (fig. 1). The minimum and maximum percentages of the different types in the blood of the mice of this series are as follows:

	Per cent
Unbroken ring,	2 to 4
Broken ring,	18 to 22
Segmenting ring,	10 to 26
Knot- or loop-shaped,	46 to 60
Multilobular,	4 to 8

The cells of the blood containing all of these types of nuclei give a strong indophenol oxidase reaction.

A differential count of the cells of the bone-marrow on the basis of the type of nuclei gives an entirely different relationship between the several forms of polymorphonuclears. From 25 to 50 per cent of the marrow cells are myelocytes. These cells have not been seen in blood smears from normal mice, although they are present in very great numbers both in the blood and in the infiltrations of the viscera in myelogenous leukemia. Hence in making a comparative differential count of the polymorphonuclears in the blood and bone-marrow it is necessary to neglect the myelocytes in the smears of the latter. The percentages of the different types of polymorphonuclear leucocytes in the bone-marrow are shown in the following table:

	Per cent
Unbroken ring,	46.7
Broken ring,	10.0
Segmenting ring,	5.3
Knot- or loop-shaped,	34.7
Multilobular,	3.3

The fact that the knot- or loop-shaped nucleus is the most common form in the circulating blood and the next most common form in the bone-marrow would seem to indicate that this is the most frequent type of transformation of the primary ring-shaped nucleus in the process of maturing.

SUMMARY

1. The percentage of lymphocytes in mouse blood equals and usually exceeds that of the polymorphonuclears.

2. The cells of the bone-marrow which give rise to polymorphonuclear leucocytes are large mononuclear cells with

very finely granular cytoplasm and apparently correspond to the myelocytes of human bone-marrow except that they are less distinctly granular.

3. In the development of polymorphonuclear leucocytes from these parent cells, an opening forms in the center of the nucleus which then becomes shaped like a thick ring or a doughnut. The percentage of unbroken ring nuclei in the bone-marrow is from ten to twenty times greater than the percentage of the same type in the blood.

4. Leucocytes with ring- or doughnut-shaped nuclei are, therefore, the youngest and least mature forms of polymorphonuclear leucocytes in the blood of mice. Since they are found in moderate numbers in the circulating blood of normal mice, they are grouped with the polymorphonuclears, and not with the myelocytes, which are distinctly pathological types for the circulating blood.

5. In the process of aging or maturing of polymorphonuclear leucocytes the ring of nuclear material becomes broken up into fragments either with or without first forming complicated knots or loops.

6. All of the above cells classed as polymorphonuclear leucocytes give the indophenol oxidase reaction.

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THE EXPERIMENTAL PRODUCTION OF OSTEOCLASTS IN THE FROG, RANA PIPIENS

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TWO PLATES

Associated with the earlier stages of bone development in the frog occur numerous multinucleated giant cells very similar to the osteoclasts of mammalian primary bone. These osteolytic giant cells are disposed for the most part along the periphery of the primary red marrow, somewhat more numerously near the terminally advancing planes of ossification. Correlative with the accelerated osteogenesis following thyroid-induced metamorphosis, red marrow appears in relatively greater amount and osteoclasts are relatively more numerous. In the adult, red marrow occurs in the long bones only for a brief period following hibernation, the spleen having assumed the function of blood formation to the exclusion of the marrow after the earlier stages of osteogenesis.

In the course of our splenectomy experiments with frogs, Doctor Speidel and I ('23) obtained one specimen which reacted to the removal of the spleen by the development after sixty-eight days of enormous amounts of red marrow in the long bones. Three additional specimens developed lesser amounts of red marrow. Other specimens that survived the experiment by fifty days reacted either by a reversion to the larval type of hemopoiesis where blood is formed chiefly in the kidney, or by the metamorphosis of their fat-bodies and portions of the mesentery into a hemopoietic tissue resembling marrow; or in a few instances by the formation of a new spleen. In a number of instances slight epiphyseal hemopoiesis occurred associated with the reactions here noted.

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The present study deals only with the femoral marrow of the single splenectomized specimen in which the long bones became packed with hemopoietic tissue; and, for comparison, with the same marrow from a very young specimen (Rana catesbiana) in which metamorphosis was accelerated by thyroid administration ('23). It concerns itself primarily with the osteoclasts of the marrow from the adult specimen. The great abundance of the osteoclasts in this marrow is obviously a part of the reaction to splenectomy, and would seem to be interpretable in terms of an effort to enlarge the medullary area for the proper accommodation of the required amount of hemopoietic marrow. Moreover, the central point of interest here concerns the unmistakable origin of numbers of these osteoclasts by process of fusion of hemoblasts, rather than by fusion of reticular cells and osteoblasts according to the general mode. The special value of this experimental marrow inheres in the indirect evidence it contributes regarding a genuine osteolytic function on the part of the multinucleated giant cells designated 'osteoclasts.'

The material was preserved in the Zenker-formol mixture of Helly, sectioned at 5 μ, and stained with the eosin-azur combination of Giemsa.

In the splenectomized specimen the shaft of the femur is compactly filled with hemopoietically very active red marrow. Peripherally, typical hemoblasts are very numerous, many in mitosis. On the extreme margin many osteoclasts occur. Occasional osteoclasts occur also within the peripheral shell of hemoblasts, but never centrally or even beyond a narrow cortical area. The osteoclasts vary from mononuclear cells, not much larger than large hemoblasts (figs. 7 and 8), to enormous irregular plasmodia with many nuclei (figs. 10 to 12). These giant cells have one common nuclear and cytoplasmic characteristic. The definitive nuclear characteristic is most striking. It inheres in a clear nucleus, almost or quite devoid of reticulum, with a densely chromatic, sharply contrasting, spheroidal nucleolus. However, between this typical osteoclast nucleus (figs. 7 and 8), and the typical hemoblast nucleus of vesicular character with a delicate granular reticulum and a chromatic spheroidal nucleolus (fig. 2), all gradations can be found (figs. 3 to 6).

As regards the cytoplasm, this is typically acidophilic in the later stages. Here also a closely graded series of transition stages can be found between the definite basophily of the hemoblast cytoplasm and the oxyphily of that of the osteoclast. Numerous examples occur where the different cells of partially fused groups of hemoblasts exhibit severally the chief phases in the series of nuclear and cytoplasmic changes leading from hemoblasts to osteoclast (fig. 1). The evidence seems conclusive that in this specimen, under the conditions of experimental splenectomy, many osteoclasts arise to a large extent by a process of fusion of originally discrete hemoblasts. Such osteoclasts may subsequently receive contributions from reticular cells (fig. 12). Possibly some develop here also, as is more generally the case normally, by the aggregation and fusion of connective-tissue cells and osteoblasts. It seems clear also that in this experimental marrow certain hemoblasts differentiate directly into mononuclear osteolytic giant cells, without fusing with larger multinuclear masses (figs. 6 to 8).

No evidence appears of either mitotic or amitotic nuclear division. The frequently binucleolate condition of the osteoclast nuclei may possibly signify an abortive attempt at amitotic division. The polynucleated condition of the typical osteoclasts is the result, at least predominantly, of cell fusions, not nuclear divisions. It would seem that the peculiar conditions of the experiment, which imposed the necessity of extensive conversion of the reticular stroma into hemoblasts, preempted the available connective tissue, and thus enforced the development of osteoclasts required for the enlargement of the diaphyseal space for the accommodation of the expanding mass of marrow upon the only cells present at a low level of differentiation, namely, the primitive hemoblasts.

Conditions in the femur of the thyroid-metamorphosed frog during early postlarval stages are very different. Here also multinucleated osteoclasts are very abundant. They have the characteristic nuclear morphology already described (figs. 13 to 16). However, their cytoplasm is in general more or less basophilic. In this marrow the osteoclasts clearly form by fusions of young connective-tissue cells, with some additions from osteoblasts. Here also nuclear increase results apparently only from new accretions of cells, not from nuclear divisions. These 'osteoclasts' are closely applied both to the primary bone and to the disintegrating (receding) cartilage. No discernible morphologic or developmental distinction appears between the chondroclasts and the osteoclasts. There is no evidence that the chondroclasts grow by additions of freed cartilage cells, as maintained by Kingery ('24). Both osteocytes and cartilage cells for the most part suffer disintegration at the time of liberation into the primary marrow areolae. Furthermore, neither globules of resorbed bone or cartilage, nor indeed ingested cells or débris of any sort, can be found in these cells, such as have been frequently described in the osteoclasts of mammalian marrow (Arey, '20; Jordan, '18 and '21; Kingery, '24; et al.). Nor can anything similar to the striated border, on the surface applied to the bone, described by Arey ('20) and others, and interpreted as a resorptive phenomenon, be discerned in these specimens. It seems more likely that this 'striate border' is indicative of local disintegration.

DISCUSSION

In an earlier study (Jordan, '19) of the posthibernation femoral marrow of the frog, where special search was made for polynucleated giant cells, nothing in the nature either of hemogenic ('megakaryocytes') or osteolytic ('osteoclasts') giant cells could be found. Granting that a few may have been present somewhere in the shaft of the femur, they were certainly very rare. Accordingly, the great abundance of the osteoclasts in the postsplenectomy femoral marrow is somehow correlated with the appearance of hemopoietic marrow compensatory to the removal of the chief organ of blood formation, the spleen. This correlation probably inheres in a demand for a larger diaphyseal space to accommodate properly the enlarging mass of the required hemopoietic marrow. This interpretation assumes an osteolytic function on the part of these giant cells—a point which is still under dispute and must next be considered.

The very fact that new osteoclasts appear in great numbers under the conditions of this experiment, where there results a development of a relatively enormous amount of hemopoietic marrow in the place of a normally fatty marrow, speaks in favor of an osteolytic function on the part of these new-formed giant cells. Of course they might conceivably be the result, or concomitant, rather than the cause of an osteolytic action. This is essentially the position taken by Arey ('20), who interprets the so-called osteoclasts as fused masses of 'depleted' osteoblasts. The close correspondence between these cells, developmentally and morphologically, and the foreign-body giant cells of pathologic foci, where a phagocytic activity is more clearly evidenced, contradicts this suggestion. Kingery ('24) suggests that a genuine osteolytic function of these giant cells is correlated with the liberation of a lytic enzyme coincident with their disintegration, which hypothetical enzyme is assumed to assist in the liquefaction and solution of cartilaginous and osseous matrix. He interprets the formation of osteoclasts as a result of degenerative processes occurring in cartilage and bone, and he derives these cells from degenerating cartilage and bone cells set free by liquefaction of cartilage and bone.

The evidence from this material, more particularly that from the early postlarval specimen of a frog whose metamorphosis had been accelerated by thyroid administration, contradicts the ascription of a condition of senility to the newly formed osteoclasts. Originally, that is after initial fusion, both nucleus and cytoplasm have the typical characteristics of healthy connective-tissue cells and lymphocytes. Very soon after fusion, however, the nucleus becomes characteristically clear; the nucleolus in consequence becomes very prominent, and the cytoplasm grows gradually less basophilic. The nuclei henceforth have the peculiar 'bird's-eye' appearance. Subsequently they may suffer intracellular resorption. These characteristics are those generally correlated with degeneration, and it must be admitted that the giant cells rapidly enter upon phases of disintegration. But this is perhaps only what should be expected in a cell active in the degree required for the rapid destruction of bone or cartilage. The occasional apparent fusions with freed cartilage and bone cells is more correctly interpreted in terms of phagocytosis.

In the femoral marrow of the larval frog the osteoclasts arise from fusions of connective-tissue cells. To a very small extent secondary contributions involve osteoblasts. Here these giant cells are associated with both cartilage resorption and bone resorption. Cartilage resorption apparently proceeds also in large part directly under the influence of connective-tissue cells. It would seem as if something might be gained in the way of efficiency and speed by the fusion of originally discrete resorptive phagocytes into larger syncytial masses. This is Mallory's ('14) interpretation of the fusion of endothelial leucocytes in the formation of foreign-body giant cells and osteoclasts.

Of primary interest in this connection is the fact that the resorptive process may be consummated by a variety of apparently very different cells: connective-tissue (reticular) cells, osteoblasts, lymphocytes, endothelial leucocytes, or hemoblasts. The tissue is apparently capable of utilizing to the same end any one of several available types of cells. In the marrow of the splenectomized frog, hemoblasts abound; the connective tissue is very sparse, having been used up in the differentiation of lymphoid hemoblasts. In this tissue, therefore, hemoblasts fuse to form osteoclasts. In the marrow of the early postlarval frog, the connective tissue predominates; lymphocytes are relatively scarce. Here osteoclasts are produced by fusions of young connective-tissue cells. In older osteogenic stages, as previously described for reptiles and mammals ('18), osteoclasts arise largely by fusions of osteoblasts.

The key to a reasonable interpretation of this varied genesis of osteoclasts may be found in the close genetic relationship among the progenitors. The mesenchyme is the mother tissue of the reticular marrow cells, lymphocytes, hemoblasts, endothelium, and osteoblasts. Lymphocytes and reticular cells, like the primitive mesenchyme, have multiple developmental potentialities. Their specific differentiation is the result presumably of the operation of specific stimuli. Both osteoblasts and lymphocytes arise from the reticular stroma and appear morphologically identical. It is only when such a cell becomes applied to a spicule of calcifying cartilage or developing bone that it may become flattened, and become distinguishable as an osteoblast. Subsequently, as it becomes trapped within the mass of forming bone, it functions as a bone cell. Thus reticular cells, lymphocytes, hemoblasts, and osteoblasts are genetically closely related. They are, moreover, only slightly differentiated. Dedifferentiation is presumably easy, and any or all of these cells are utilized in the formation of osteoclasts, depending upon their relative numerical availability. The fact, then, that hemoblasts can function as osteoclasts, either singly or in fused groups, is not as startling as it at first appears. As a primitive lymphocyte the hemoblast is endowed with multiple developmental capacities. The stimulus for osteolytic function is met by the proper reaction by the only adequately available cell, and osteoclasts arise from hemoblasts.

It was stated above that a number of osteoclasts were located within the peripheral layer of hemoblasts, not on the margin next the bone. In general these deeper-lying osteoclasts are in early stages of disintegration, as indicated by a certain amount of peripheral fragmentation, and larger or smaller areas of chromophobic cytoplasm. The intervention of masses of hemopoietic marrow between the diaphyseal bone and certain of the osteoclasts may be the result of the invasion by marrow of the spaces excavated from the bone by the osteoclasts. In the early postlarval marrow the osteoclasts are all immediately applied either to cartilage or bone.

The evidence from these two specimens of marrow supplies almost a demonstration of the resorptive activity of these giant cells. In their earliest stages they show no marks of degeneration. The early onset and rapid progress of degeneration and disintegration is explicable in terms of the rapidity and intensity of the lytic process. In this they resemble the monocytes of the blood and tissues. When intense phagocytosis is forced upon these cells, as by the injection of India ink into the dorsal lymph sac of the frog, they soon become loaded with the carbon particles to an extent that may even cause rupture. Concomitant with the loading process, the cytoplasm becomes greatly vacuolated (Jordan, '24).

In an earlier contribution to this subject I ('21) described a special instance, that of the jaw of a newborn cat, in which certain osteoclasts contained variable globules apparently identical with bone. This case still remains unique; and until other similar instances are discovered, the interpretation given must be considered tentative. However, it was originally stated that while the material apparently definitely demonstrates an osseophagic function on the part of the osteoclasts, this particular mode of osteolysis must be rare; the more usual mode of the presumed bone resorption being one of prior complete liquefaction of bone matrix. That osteoclasts have at least a certain degree of phagocytic capacity is demonstrated by their occasional content of red blood corpuscles and the nuclei of freed and disintegrating bone and cartilage cells. If, therefore, bone were available in the form of small semifluid globules or even solid fragments, there is no reason why it should not be phagocytosed as such. However, bone dissolution does not generally involve such elements. Whatever the source of the lytic enzyme, whether osteoclast, as seems more likely, or blood or connective tissue or still other elements, the disappearing bone is in non-solid form. That it may be absorbed by the osteoclasts in more or less fluid condition is perhaps indicated by the vacuolated condition of the cytoplasm of the osteoclasts, especially in young bone where resorption proceeds very rapidly (fig. 14). Furthermore, the gradual loss of basophily in the osteoclast cytoplasm, occurring first locally, may be the result of the same process involving the admixture of resorbed fluid. This local clarification of the cytoplasm is due in part also to the dissolution and resorption of certain nuclei.

SUMMARY

1. The femoral hemopoietic marrow of the frog, formed as a compensatory reaction to the experimental removal of the spleen, contains peripherally large numbers of multinucleated giant cells. These cells arise chiefly by the fusion of hemoblasts; secondarily, a few stromal cells may become added. Osteoclasts are generally absent or very rare in normal marrow of adult frogs. The only reasonable interpretation of the great increase in number of the osteoclasts in this experimental marrow, namely, as a response to a demand for greater medullary area for the proper accommodation of an enlarging mass of red marrow in the absence of the spleen, is in accord with the ascription of an osteolytic function to the so-called osteoclasts.

2. The femoral marrow of young frogs whose metamorphosis was accelerated by thyroid administration is very rich in polynucleated giant cells. Here they arise almost exclusively by fusion of the stromal cells, small secondary additions involving osteoblasts. They function both as chondroclasts and osteoclasts.

3. Osteoclasts may originate by fusion from several types of cells: reticulum cells, lymphocytes (hemoblasts), osteoblasts, and even in small part freed bone and cartilage cells, and possibly also from endothelial cells. They may be derived from only one type of cell, or by combinations of several types. The prevailing mode of origin follows the predominating cell type. In the adult experimental marrow,

where the hemoblast dominates, the reticular cells having been used up as hemoblast progenitors, osteoclasts arise chiefly from hemoblasts. In the early postlarval marrow, where mesenchyme is still plentiful, the osteoclasts form largely by fusion of stromal cells. Subsequently, when osteoblasts are abundant, osteoclasts may originate in part from these cells. The varied origin of osteoclasts at different stages of bone development is explicable on the basis of the close genetic relationship among the cells involved, and their relatively low degree of differentiation.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 to 12 Cells from hemopoietic marrow of femur of frog, sixty-eight days after total splenectomy. Helly fixation, Giemsa stain. × 1200.

1 Osteoclast in process of formation by fusion of eight hemoblasts, three still partially separate.

2 Typical hemoblast.

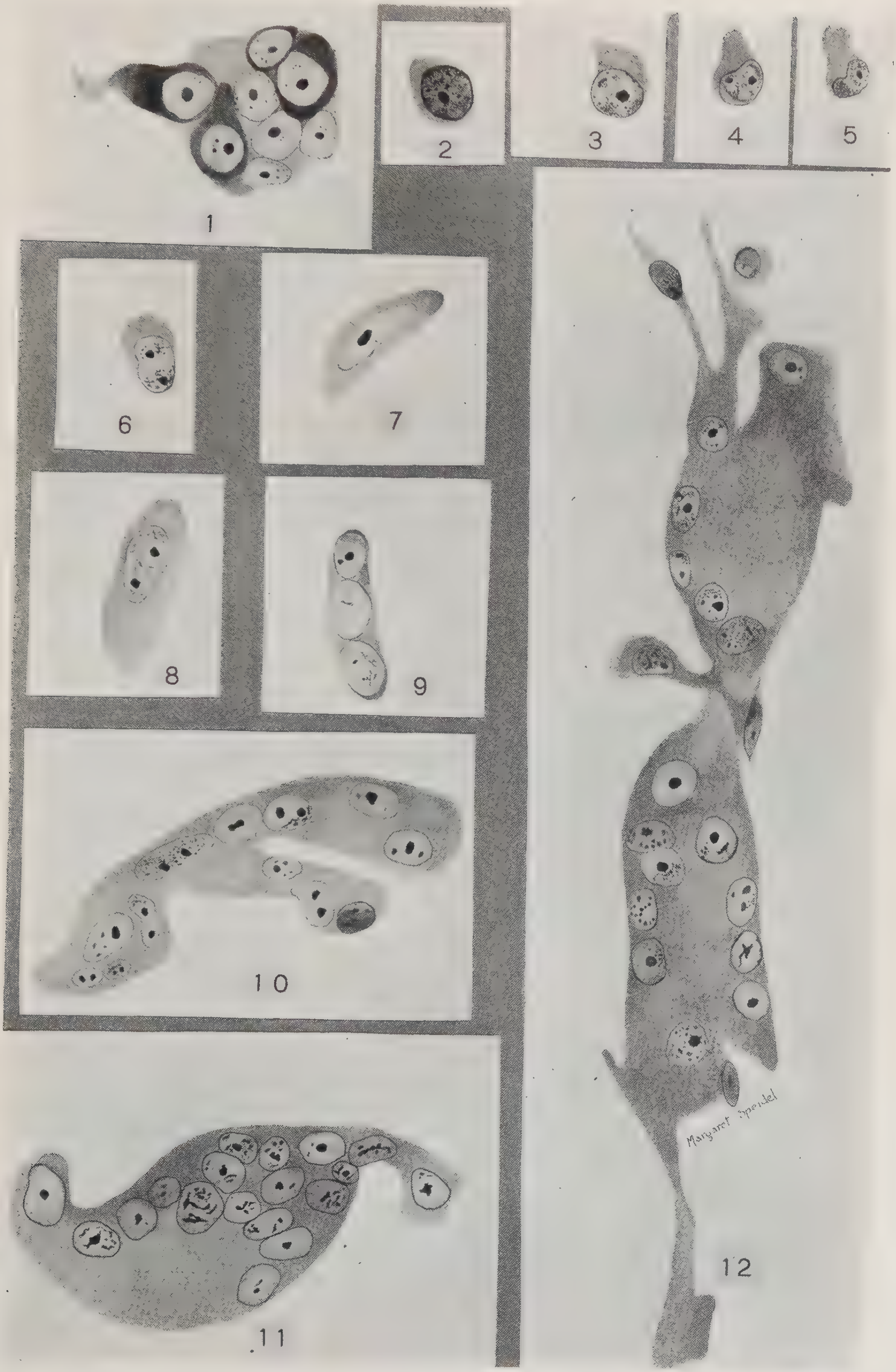
3 to 7 Stages in the transformation of a hemoblast into a mononuclear osteoclast.

8 Large mononuclear `osteoclast.

9 Trinucleated osteoclast.

10 and 11 Two large, irregular, multinucleated osteoclasts. Variations in size and shape are almost limitless.

12 Huge osteolytic giant cell (osteoclast), showing secondary stromal additions, and the beginning of disintegration along the lower end.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Margaret Spendel

PLATE 2

EXPLANATION OF FIGURES

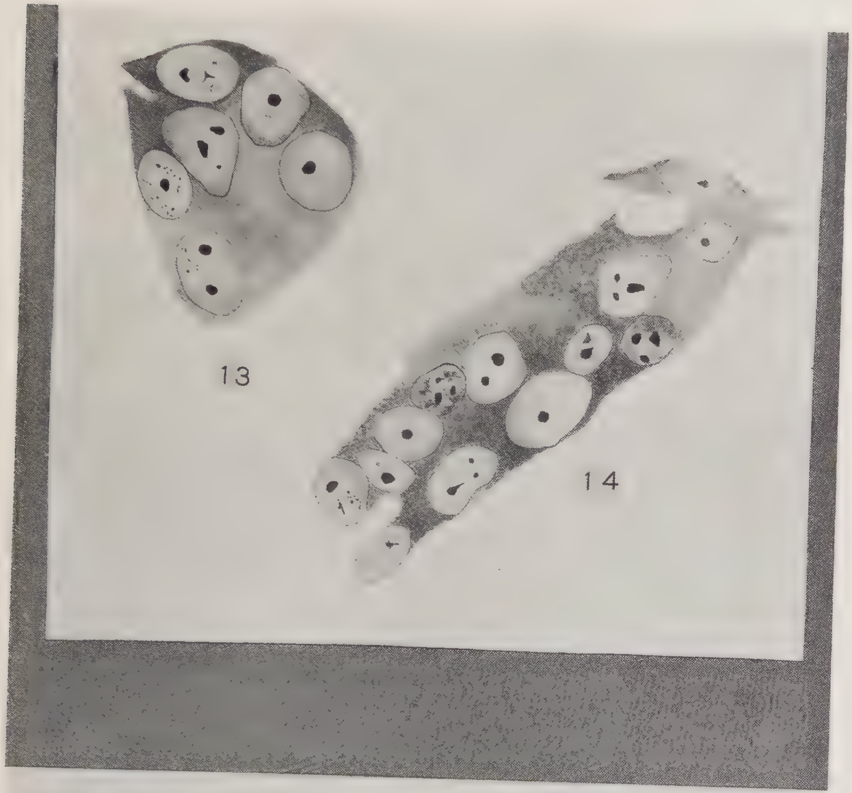
13 to 16 Osteoclasts from the femoral marrow of an early postlarval specimen whose metamorphosis was hastened by thyroid administration. Helly fixation, Giemsa stain. × 1200.

13 Osteoclast in process of formation by fusion of stromal cells, the upper cell, next the receding bone, still partially separate.

14 Similar, somewhat older osteoclast. This cell adjoined the bone along its left border. Along this border the cytoplasm has a vacuolated, coarsely granular structure.

15 Osteoclast still in connection with the medullary stroma above. Below, it adjoins bone. Along the projection at the right, it touches disintegrating cartilage.

16 Small area of medullary stroma adjacent to cartilage at the left, including one osteoclast. This portion of the cartilage includes one cell, below at the left. The cartilage is dissolved in part by the action of unfused stromal cells. As the freed cartilage cells drop into the new-formed marrow spaces, they are little more than naked nuclei, and rapidly suffer disintegration. An occasional cartilage cell may fuse with, or be phagocytosed by, the adjacent osteoclasts.



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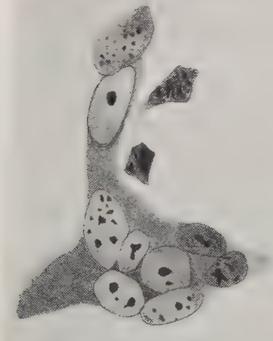

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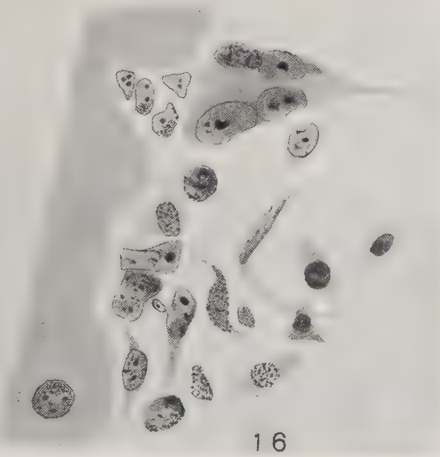

16

SIMPLE APPARATUS FOR SAWING AND GRINDING SECTIONS OF BONE AND TEETH

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SIX FIGURES

The sawing and grinding by hand of sections of teeth and bone for microscopical study is exceedingly laborious and the results of the process are, to say the least, uncertain. An excellent device for the mechanical preparation of polished sections of calcareous tissues has been developed in connection with dental research (for a description see Black, in Noyes and Thomas' Text-book of Dental Histology, Lea & Febiger, 1921). This apparatus, however, can be procured only made to order and, for most laboratories, its cost is prohibitive. A home-made apparatus for such work has been constructed in this laboratory, which, although it cannot be operated with quite the nicety of that described by Black, yields satisfactory results for all ordinary purposes. Furthermore, it is inexpensive and can be constructed in the ordinary laboratory workshop by anyone with a modicum of mechanical ability.

Our apparatus consists of two distinct units—a device for the preliminary sawing of thin slices of calcareous or siliceous objects (Chase) and a device for the grinding and polishing of these slices to the thinness necessary for microscopical study (Patten).

The general arrangement of the apparatus may readily be grasped from figure 1. Both units and the motor are mounted on a substantial table which has felt pads affixed to its feet to minimize vibration. A 1/6 H.P. motor of about 2300 R.P.M.

will furnish ample power. A table top of approximately 2½ by 3½ feet affords sufficient area for mounting all parts of the apparatus without crowding.

THE SAWING APPARATUS

The sawing apparatus consists of a motor-driven disc of aluminum and a device which permits orienting the specimen accurately, holding it rigidly, and advancing it uniformly against the edge of the rotating disc. The specimen is cut by the abrasive action of powdered carborundum fed in a soapy suspension to the edge of the disc.

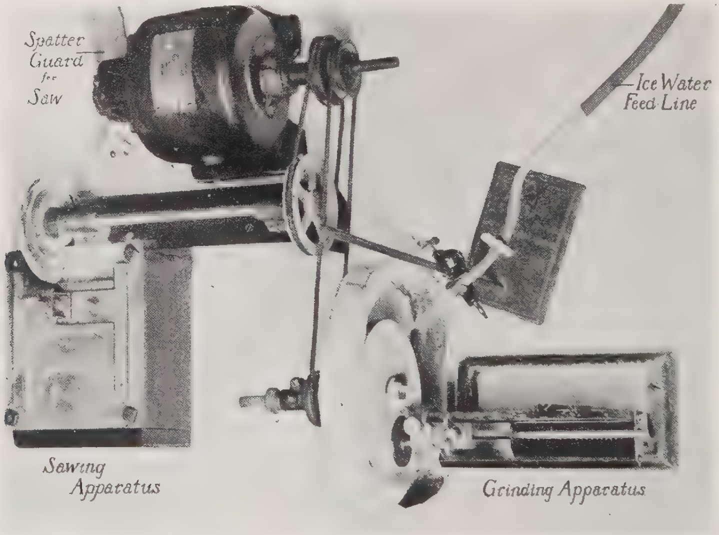

Fig. 1 Apparatus, photographed from above, to show general arrangement.

We use a disc 6 inches in diameter, cut from 28-gauge sheet aluminum. The disc is mounted between 3-inch steel washers (for rigidity) on an arbor at the end of a motor-driven shaft (fig. 1). The pulley on the shaft should be somewhat greater in diameter than that on the motor. We use a 4-inch pulley on the shaft and a 2½-inch pulley on the motor. Before use, the cutting disc must be trued and smoothed by a fine, flat file held lightly against its periphery as it revolves. Such a disc will cut hundreds of sections before it wears to a diameter too small to be of further service.

The holding and feeding device must provide both for advancing the object to be sawed against the edge of the cutting disc and for moving the object across the plane of the disc for cutting successive sections. This necessitates the provision of two slides working at right angles to each other, one movable in an axis parallel to, the other in an axis perpendicular to the plane of the disc.

Both slides, and the object clamp which they carry, are mounted on a base plate of brass supported on oak blocks and bolted rigidly, through the blocks, to the top of the table. The base plate is slotted to receive the aluminum cutting disc and its arbor washers (figs. 1, 2).

The slide which moves in an axis parallel to the plane of the disc ('sliding carriage,' figs. 2, 3) is mounted on the base plate in a suitable track. The plan of construction and the dimensions of the various parts of the track assembly are shown in figures 2 and 3. Only one feature needs further comment. The sliding carriage must not bear directly on the base plate, because, in the presence of grit thrown by the cutting disc, such an extensive area of contact would cause too much friction with resultant uneven operation and jamming of the sliding carriage. Difficulty due to the presence of grit cannot be entirely obviated, but it is minimized by inserting a member in the track assembly which raises the sliding carriage slightly above the base plate, where the grit tends to collect, and provides it at the same time with a greatly reduced bearing surface (fig. 3). When the sliding carriage has been fitted so that it works smoothly in the track assembly, a slot is cut in it to receive the disc and its washers. This slot must correspond in shape and position to the slot in the base plate (fig. 2).

The slide which operates at right angles to the plane of the disc we have termed the 'transverse slide.' To raise the transverse slide so that it will clear the track assembly of the sliding carriage, it is mounted on a small base plate of its own ('base for transverse slide,' figs. 3, 4). The transverse slide moves in beveled tracks which are screwed to the sliding carriage, the screws passing through the base for the transverse slide in order to hold both the tracks and the base firmly in place.

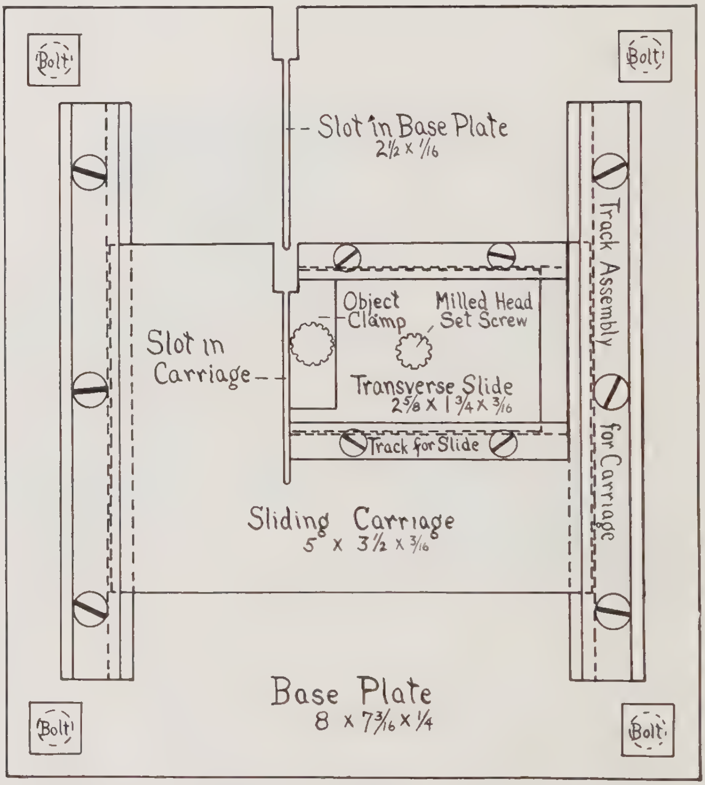
Bolt
Bolt
Slot in Base Plate
2½ x 1/16
Track Assembly for Carriage
Object Clamp
Milled Head Set Screw
Slot in Carriage
Transverse Slide
2⅝ x 1¾ x 3/16
Track for Slide
Sliding Carriage
5 x 3½ x 3/16
Base Plate
8 x 7 3/16 x ¼
Bolt
Bolt

Fig. 2 Plan of sawing apparatus, as seen from above. The dimensions given are in inches.

The transverse slide has mounted on it an object clamp suitable for holding and orienting a fiber block to which the specimen to be sawed may be affixed. The clamp we are using was taken from an old microtome and allows adjustment of the block in the horizontal plane only. This clamp gives satisfactory service, but one which allowed rotation in more than one plane would be more efficient.

The transverse slide is further provided with a milled-head set-screw, by means of which the slide may be held rigidly in place after the orientation of the object has been accomplished. A scale with divisions in millimeters can be scratched on the edge of the transverse slide, or on the track for this slide, for convenience in gauging the thickness of the section to be sawed.

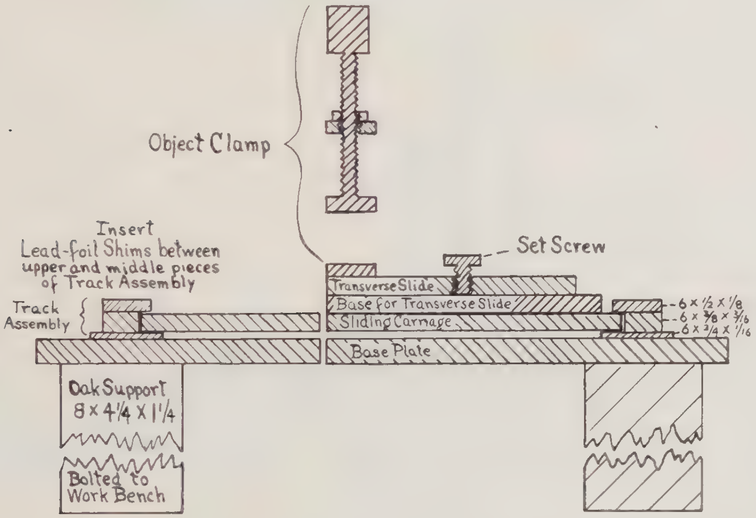
Object Clamp
Insert Lead-foil Shims between upper and middle pieces of Track Assembly
Track Assembly
Set Screw
Transverse Slide
Base for Transverse Slide
Sliding Carriage
Base Plate
6 × 1/2 × 1/8
6 × 3/8 × 3/16
6 × 3/4 × 1/16
Oak Support 8 × 4 1/4 × 1 1/4
Bolted to Work Bench

Fig. 3 Sectional plan of sawing apparatus. The plane of section passes vertically through the middle of the transverse slide and at right angles to the slot for the cutting disc (fig. 2). The dimensions given are in inches. The specifications for the oak supports are for those used in our apparatus. Their height is dependent on the type of clamp used and on the height at which the shaft of the cutting disc is mounted. The sawing apparatus should be mounted at such a height that the object to be sawed is at the level of the center of the disc.

A spatter guard must be provided for the cutting disc. This can easily be constructed from a sheet of galvanized iron or zinc. It should be sufficiently large to extend from a hinge on the table back of the sawing apparatus to a point just clear of the near edge of the sliding carriage. It can conveniently be semicircular with a radius sufficiently long to allow it to clear the disc and the top of the object clamp.

For our apparatus the spatter guard was made from a piece of galvanized-iron sheet 20 by $2\frac{1}{2}$ inches. Cuts to a depth of $\frac{1}{2}$ inch were made on both sides at intervals of 2 inches. The flanges were then bent at right angles and the guard bent to form a semicircle, the overlapping edges where the cuts were made being soldered to add rigidity and prevent leakage through the flange. Hinging the guard permits it to be thrown out of the way during preliminary adjustments.

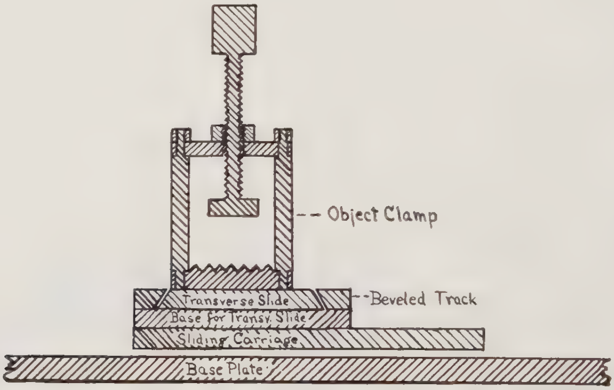

Fig. 4 Sectional plan to show arrangement of transverse slide and object clamp. The plane of section passes through the object clamp and parallel to the slot for the cutting disc (fig. 2).

THE GRINDING APPARATUS

Like the sawing apparatus, the grinding apparatus may be considered under two heads—the motor-driven grinding wheel and the device for the controlled advance of the object against the wheel. Each part presents certain very definite requisites. The wheel must be of the proper type and grade of abrasive; it must be driven at the proper speed; and above all it must have a true grinding face. The device for advancing the object against the grinding face of the wheel must insure an accurate and rigidly maintained alignment of the object to the cutting face of the wheel, a uniform rate of feed toward the wheel at correct pressure, and an accurate means of checking the grinding when it has progressed to the desired point.

The grinding-wheel assembly is the first part of the apparatus to be set up. Its position with reference to the motor, the sawing device, and the mechanism for advancing the object to be ground is shown in figure 1. The wheel is mounted on a grinding head which has a base drilled for bolts so that it can be securely fastened to the table top. We are using one with an arbor at each end of the shaft (Goodell-Pratt Co., Catalogue 14, no. 25). One arbor is all that is needed in connection with the grinding apparatus, but the extra arbor affords a place for setting up a coarser stone or a small circular saw for purposes of general utility without disturbing the grinding apparatus.

In grinding wheels there is a wide variety to choose from. A size of 6 inches in diameter and ½ inch in thickness is convenient. The abrasive should be fine. Carborundum wheels of about '220 grit' cut very rapidly and work well unless very thin sections are desired. For thin sections the 'carborundum flour' wheels (about grade 2F.) are preferable, although they do not cut as rapidly. For exceedingly delicate work Black recommends an Arkansas white-stone wheel, but they are quite expensive, and we have been able to get entirely satisfactory results with the much cheaper carborundum wheels.

The grinding head should be fastened to the table, connected to the motor by a belt (using a pulley on the motor of the same diameter as that on the grinding head), and the wheel 'dressed' before proceeding with the remainder of the apparatus. The grinding surface of the wheel can be dressed to a true face most satisfactorily by a good mechanic with a lathe at his disposal. It is possible, however, with the exercise of care and patience, to true the wheel on the grinding head on which it is to be run. This is done by bringing a large carborundum crystal, in a holder supported by a tool rest, against the grinding surface while the wheel is being driven by the motor. Great care must be used to secure a steady rest and to avoid making concentric grooves in the face of the wheel. When the high spots have been dressed down by this method, the wheel is taken off the arbor, laid flat on a bench, and the grinding surface rubbed smooth with a large, coarse carborundum hone which has a true face.

The next step is to assemble the device for advancing the object against the grinding wheel. The basis for this part of the apparatus is a discarded sliding microtome—an article which can be found in almost any laboratory. The size or type of the microtome makes but little difference, since the essential requirement is a feature common to all types—a heavy metal block (the knife carriage or sliding carriage) moving in an accurately milled track. On the sliding carriage, instead of the knife, is mounted a clamp for holding an object disc. This entire assembly is actuated by a spring to feed toward the grinding surface of the wheel (fig. 5). A micrometer check screw which stops the advance of the carriage at any desired distance away from the wheel enables the operator to control the thickness of the section.

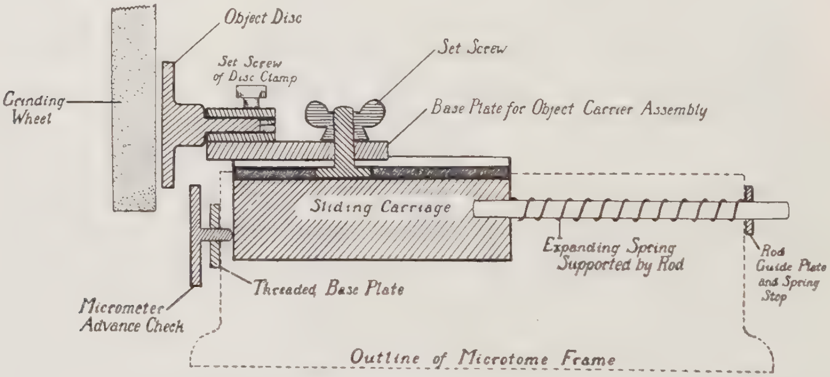
Object Disc
Set Screw
Set Screw of Disc Clamp
Grinding Wheel
Base Plate for Object Carrier Assembly
Sliding Carriage
Expanding Spring Supported by Rod
Rod Guide Plate and Spring Stop
Threaded Base Plate
Micrometer Advance Check
Outline of Microtome Frame

Fig. 5 Sectional plan of the automatic feeding mechanism of the grinding apparatus. The plane of section is vertical and in the long axis of the converted microtome (cf. fig. 1).

The following procedures are involved in the conversion of the microtome. One end of the knife carriage is drilled and tapped to receive a correspondingly threaded piece of $\frac{1}{4}$-inch brass rod which serves as a guide for an expanding spring. Across the end of the microtome frame is fastened a brass plate in which a 9/32-inch hole has been cut to receive the guide rod. This plate acts also as a stop for the spring (figs. 1, 5, and 6). We now have the old microtome frame constituting a substantial and smoothly working track in which the carriage slides toward the grinding wheel due to the expansion of the spring. The pressure exerted by the spring should be sufficient to move the carriage along the track with no danger of its sticking, but at the same time it should not be excessive. Too strong a pressure of the object against the wheel is likely to result in damage to the sections. The exact strength of spring desirable would vary with the weight of the carriage on different microtomes, but the following specifications for our own spring may serve as a general guide. It is made of steel wire 1 mm. in diameter. Uncompressed, it makes one and one-half turns to the centimeter. It is compressed one-fourth of its length at rest when it is set in position. When the sliding carriage is in the position it occupies during grinding (fig. 5), the spring exerts sufficient pressure to overcome the friction of the carriage on the track and raise a 500-gram weight attached to the carriage by a string and pulley.

A calibrated micrometer screw for controlling the thickness of section is ready to hand in the micrometer screw which was used to advance the object carrier toward the knife of the microtome. This screw, together with the plate into which it is threaded, can readily be removed from its old position and, by drilling and tapping new screw holes, can be affixed to the end of the microtome bed in the position indicated in figure 5, where it appears under the name of 'micrometer advance check.' The position in which the plate is attached is similar to that shown in figure 6 for the guide plate of the spring rod, but it is located at the opposite end of the microtome. By adjusting this micrometer advance check the distance to which the spring-actuated sliding carriage can travel toward the grinding wheel is controlled. This makes it possible to set the apparatus so that grinding will cease automatically at any desired thickness of section.

For convenience in reading the graduations on the head of the micrometer advance check a pointer should be affixed to the frame of the microtome.

For fastening the object-disc clamp to the sliding carriage, one part not furnished by the microtome is necessary. This part is shown in figures 5 and 6 under the name of 'base plate for object carrier assembly.' Its exact dimensions are not given, as they would vary depending on the type of the microtome used. The part in question consists simply of a piece of brass about 2 inches long by 1 inch wide and not less than ¼ inch in thickness. The bottom of this plate must be milled or filed to form a tongue fitting into the groove in the top of the sliding carriage (fig. 6). This tongue should extend throughout the length of the base plate and fit fairly snugly, as upon it depends the rigidity with which the alignment of the entire object-carrier assembly is maintained. One end of this plate is drilled to receive the bolt and its winged set-screw formerly used to hold the knife on the carriage. To the other end is brazed (or affixed with machine screws)

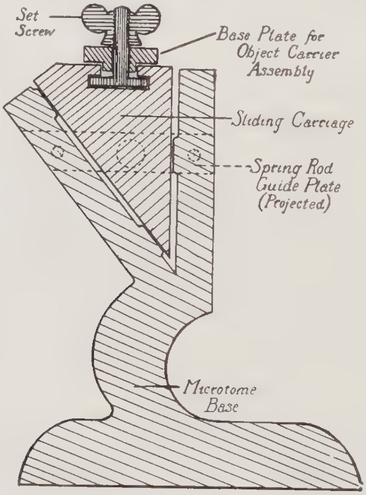
Set Screw
Base Plate for Object Carrier Assembly
Sliding Carriage
Spring Rod Guide Plate (Projected)
Microtome Base

Fig. 6 Cross-section of automatic feeding mechanism through sliding carriage and base plate for object carrier assembly.

the clamp for the object disc (fig. 5). Great care should be taken that this clamp be set in perfect alignment, otherwise the sections ground will not be of even thickness throughout.

When the grinding wheel has been set up and trued, and the advancing mechanism assembled, the final step is to align the advancing mechanism so that the face of the object disc is exactly parallel to the grinding surface of the wheel. Then bolt the base of the advancing mechanism to the table. Before the nuts are fully tightened, it is well to test the alignment. Start the grinding wheel and then, by slowly unscrewing the micrometer advance check, allow the object disc to advance toward the wheel until it just touches. By repeated trials, noting each time the way in which the object disc is marked by the wheel, it is possible to perfect the alignment of the entire apparatus. The nuts on all bolts holding the various parts of the apparatus to the table should then be fully tightened. The use of lock washers is advisable to guard against the loosening of nuts due to the vibration caused by the motor.

Since the grinding must be done under a constant flow of ice water, a spatter guard for the grinding wheel is necessary. It is made in the same general manner as that for the saw, already described. The guard for the wheel, however, must completely encircle it and provide an outlet at the bottom to carry off the accumulation of water.

Ice water may conveniently be fed to the wheel from a glass tube drawn out to a caliber of about 1 mm. The nozzle is supported by a clamp attached to a ring stand. A pinch clamp on the rubber tubing leading from the water container affords control of the stream. Any convenient container may be used, but one with a generous capacity avoids the nuisance of frequent refilling.

USE OF THE APPARATUS

Specimens of teeth or bone for grinding should, immediately on removal from the body, be immersed in fluid, preferably a fixing fluid. Throughout their entire preparation they should never be permitted to become dry. Teeth especially, which have once been allowed to dry, are exceedingly friable, and it is next to impossible to secure complete thin sections from them.

In order to obtain more than one section from a specimen it is necessary to saw it into slices which may subsequently be ground. Some objects can be held for sawing directly in the jaws of the clamp. Objects which cannot be so held we mount on pieces of red fiber block in one end of which appropriately shaped holes or grooves have been cut to receive that part of the object which is to be affixed to the block. The other end of the block is so shaped as to be grasped firmly by the jaws of the clamp. The object is cemented in the desired position by means of sealing wax, which holds it securely, provided the wax is built up somewhat about its sides. It is possible to avoid drying the object by mounting from 95 per cent alcohol, blotting only the superficial alcohol from the area which is to be affixed to the block. The block bearing the object is then kept wet with water (alcohol dissolves sealing wax) until the desired sections have been cut from it.

During the operation of sawing the right thumb pushes gently and evenly on the middle of the near edge of the sliding carriage. Meanwhile the left hand applies the soapy suspension of powdered carborundum to the edge of the disc, immediately above the object, by means of a water-color brush of a size at least as large as no. 10. Care must be taken to apply the carborundum to both faces of the edge of the disc, so that it will abrade the object in a straight scarf. The brush must be redipped frequently in the well-stirred carborundum suspension. Results are most satisfactory if the sliding carriage is advanced at such a rate that the object bears against the disc with a light, uniform pressure just sufficient to cause the abrasive to cut. Too much pressure results in binding between the object and the disc. A little practice will readily show the operator the proper speed of sawing. Enamel is sawed much more slowly than dentine or bone and must be fed especially evenly to prevent denting the edge of the disc. If the edge is dented it is necessary to smooth it with a file before further sawing is done. If one attempts to continue with even a slight imperfection, serious damage may be done to both disc and object.

As each slice is sawed, it is placed in water, in which it is rinsed and scrubbed with a stiff brush to remove the soft tissues and particles of carborundum. The surface to be affixed to the object disc of the grinding apparatus is next ground flat and polished. This may be accomplished by inserting the slice in a shallow depression in a cork and holding it, by means of the cork, against the rotating carborundum wheel. During this process, and during all subsequent grinding and polishing operations, the surface of the wheel must be supplied with a flow of ice water. The cork must be held very steadily in one position to avoid the production of a surface which is not plane. Fairly large pieces may be held against the wheel on the end of a finger, but for small slices this procedure is too destructive of epidermis. Still another method is to affix the slice to the object disc with balsam as described below, and allow it to be advanced against the wheel by the spring-actuated feeding device. This method, while more accurate, is time consuming, practically doubling the work of the grinding process. We use it only for especially valuable specimens.

When one surface of the slice has been ground plane by one of the above methods, it is necessary to adjust the apparatus preparatory to grinding the other face. This involves setting the micrometer advance check so that it will stop the grinding action when the slice has been ground to the desired thinness. It is a convenience, in grinding a large number of sections, to have available several object discs.[1] These discs are likely to vary somewhat in thickness, hence the setting of the check screw will vary for the different discs. Each disc must, therefore, be numbered and calibrated. This may be done in the following manner. The shank of the object disc is inserted in the clamp, care being taken that it is pushed in until firmly seated. Then the set-screw of the clamp is tightened. Next the micrometer advance check is unscrewed until the sliding carriage comes to rest against the 'threaded base plate' of the advance check (fig. 5). The entire object-carrier assembly is now moved into such a position on the sliding carriage that the object disc bears firmly against the grinding wheel. Then the set-screw which locks the object-carrier assembly in place on the sliding carriage is tightened and the micrometer advance check screwed in until the object disc is clear of the grinding wheel. The grinding wheel is now started rotating and the advance check turned very slowly until the object disc just 'chatters' against the wheel. The wheel is then stopped and the reading on the head of the advance check taken. The reading so obtained is the 'zero point' for this object disc—the point at which a section on the disc would have been completely ground away. From the pitch of the check screw it can readily be computed how far to turn the screw in order to stop the grinding at such a point that the desired thickness of section will be obtained. The calibration of the object discs need not be repeated each time a section is ground, provided the position of the object-carrier assembly on the sliding carriage remains fixed. The object discs must be recalibrated after the grinding of a large number of sections, however, to allow for the wearing away of the grinding surface of the carborundum wheel.

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[1] If it is not possible to buy object discs of the proper type, they may readily be turned out of brass. The face of the disc should be about 1½ inches in diameter and should be plane. The concentric grooves usually cut in object discs for mounting paraffin blocks lessen the cohesion of the object to the disc.

Before grinding the second face of the section it must be run through ascending grades of alcohol to absolute. From this medium it is affixed to the object disc by a very small amount of Canada balsam. The balsam should be thick enough to be almost solid at room temperature, but not brittle. A thin, uniform film of such balsam is spread on the surface of the previously heated object disc, the polished face of the slice dried rapidly with a jet of air, and this face pressed firmly and with a rotary motion against the still warm, balsam-covered face of the disc. As soon as the slice is in contact over its entire ground face, the object disc, with the slice held firmly and in one position against it, is plunged into cold water to cause the balsam to solidify. The 'spiders' and 'dogs' described by Black are valuable aids in affixing slices to the object disc, especially if more than one slice is to be mounted on the same disc. They are not essential for satisfactory results if only one section is to be ground at a time. As Black has indicated, the proper consistency of the balsam is of the utmost importance. It should be just thick enough to become solid, but not brittle, in ice water. If the balsam is too thin, the slice will 'creep' on the face of the object disc during grinding. If it is too thick it becomes brittle and the slice breaks from the object disc. The most desirable consistency seems to vary considerably in different lots of balsam and can be determined only through personal experience. Black states that it should be stiff enough to move sluggishly at 110°F., but fluid at 120° or 130°F.

After the slice has been affixed to the object disc, all excess balsam should be removed from the disc and section by means of a cloth moistened with xylene. This is necessary to avoid gumming the surface of the grinding wheel. The object disc is then placed in position on the grinding apparatus and the check screw set at the desired reading. The ice-water flow is started, being fed preferably toward the center of the wheel so that the whole grinding surface will be constantly covered with water. Then the motor is started and the grinding allowed to proceed automatically to completion.

When the grinding of a section is finished, the object disc bearing the section is placed in a low grade of alcohol or in water, where it is rinsed and brushed to remove the dust of grinding. Thence it is run rapidly through the higher alcohols and finally into xylene, where it must be left until the balsam used in affixing the section has been completely dissolved. When the section is freed from the disc it must again be cleaned to remove the dust which was embedded in the balsam. This may be accomplished with least risk to the section by holding it with one camel's-hair brush and brushing it gently with another.

Before mounting, the section may be treated by a variety of special methods (staining, impregnation, etching, etc.), discussion of which is out of place here. For ordinary purposes satisfactory optical differention may be obtained by mounting sections in balsam in such a manner that air remains included in the lacunae and canaliculi of bone and cementum and in the dentinal tubules and 'enamel spindles' of teeth. This may be accomplished in the following manner. The section is transferred from xylene to absolute alcohol for five minutes, thence to blotting-paper, where it is allowed to dry. (Drying directly from xylene leaves a white residue.) The balsam used must be sufficiently thick to prevent it from invading the air-filled spaces in the section. Such balsam is too thick to be handled readily at room temperature and must therefore be warmed on the slide before the section is embedded in it. Its thickness, even when warmed, makes it difficult completely to exclude air bubbles around the section and under the cover unless a liberal amount is used. To get rid of the excess balsam between the slide and cover and to flatten the section which has a tendency to warp, mounted specimens are placed for a few days in an oven at 38°C. with a weight or spring clip on the cover.

SEVEN ARTERIAL ANOMALIES OF THE HUMAN LEG AND FOOT

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FIVE FIGURES

The seven anomalies presented in this article seem of special interest in connection with Prof. H. D. Senior's recent interpretation of recorded anomalies of the human leg and foot. In his paper ('19 b) he discussed the probable origin of certain arterial anomalies which had appeared in the literature; but at that time dissected specimens were not available for this study. Since then a number of such anomalies have appeared in the dissecting-room, so that it is now possible to ascertain the exact relation of the blood vessels to the muscles—an important criterion which has not always been available in published accounts of these variations. At Professor Senior's suggestion and under his guidance, I have undertaken a description of these new cases.

The following are the only arterial anomalies of any consequence in the human leg and foot that have occurred in this department during the past fifteen years.

Three types are represented:

a. In three limbs, two of which are from the same subject, the posterior crural region and sole were supplied by one large artery: the a. peronaea magna (H. D. Senior, '19 b).

b. In three others these regions were supplied by three large arteries, one being anomalous.

c. In the remaining limb they were supplied by two large arteries, of which one was anomalous, the posterior tibial being absent.

In the cases belonging to the first group, the parts of the one large artery are derived from the following embryonic elements, as in the cases published by Bourgery and Jacob, Tiedmann, Quain, Barkow, and others, which were reviewed by Senior.

1. The part of the single artery of the leg extending from the origin of the a. genu inferior to the proximal border of the m. flexor hallucis longus represents part of the a. peronaea posterior superficialis of the embryo which is continued as a small branch to the m. soleus.

2. The part between the mm. flexor hallucis longus and tibialis posterior, and under cover of the former is the persisting embryonic ramus communicans inferior.

3. The part in contact with the posterior surface of the interosseous membrane is a derivative of the embryonic a. interossea. It gives off a ramus perforans of normal type.

4. The part which passes behind the tendon of the m. flexor hallucis longus and behind the medial malleolus into the sole consists of part of the main stem of the a. peronaea posterior superficialis. It terminates as the lateral plantar artery and a vessel resembling the a. plantaris medialis. The lateral calcaneal branch arises from the fourth part of the main artery near or together with a lateral posterior malleolar branch in the normal place. The embryonic a. coronaria malleolaris (Hyrtl) springs from the main artery at about the same level as the lateral calcaneal. According to Hyrtl (Schlagadern des Unterschenkels, '64, p. 21), the r. coronarius malleolaris of the a. peronaea originally passes medially around the tibia to join the a. tibialis anterior. Later it also anastomoses with the a. tibialis posterior. Its part between the a. peronaea and the a. tibialis posterior becomes the r. communicans; its part connecting the aa. tibiales becomes the aa. malleolares mediales and the rete malleolare mediale.

In one of the three cases the plantar arteries are smaller than usual. The foot was supplied mainly by the a. dorsalis pedis. In one case the ramus perforans a. peronaeae and the ramus calcaneus lateralis arise by a common stem. The arteries in the other regions of the extremity are normal in these specimens.

The composition of the a. peronaea magna is that of the normal a. peronaea (Senior, '19 b) except for the fourth

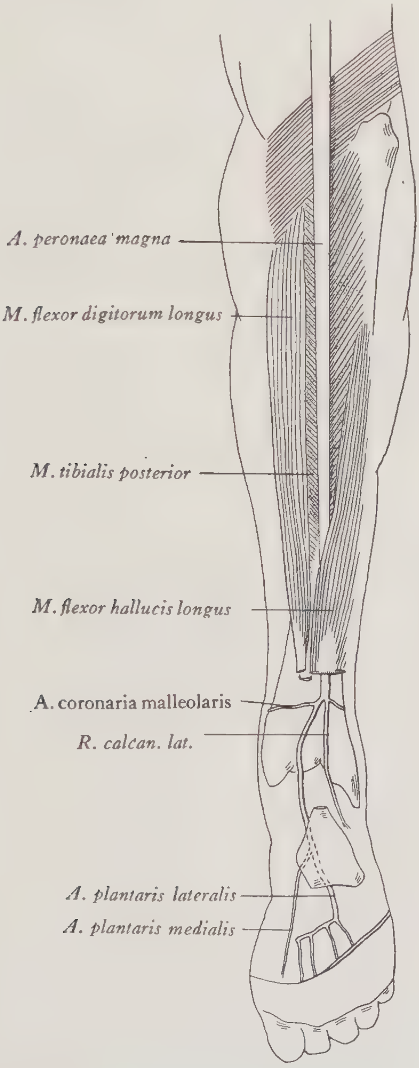

A. peronaea magna
M. flexor digitorum longus
M. tibialis posterior
M. flexor hallucis longus
A. coronaria malleolaris
R. calcan. lat.
A. plantaris lateralis
A. plantaris medialis
1

Fig. 1 The arteria peronaea magna.

part, which is continued into the sole as the a. plantaris lateralis. The lateral calcaneal branch, given off by the fourth part, follows the course of the terminal part of the normal a. peronaea.

According to Senior, the normal a. plantaris medialis may be looked upon as the persisting terminal part of the a. tibialis posterior superficialis, and the normal a. plantaris lateralis as formed by the two terminal branches of the a. peronaea posterior superficialis. The a. peronaea posterior superficialis is described by that author in an embryo of 14 mm. as bifurcating ''upon entering the sole, into a medial and lateral terminal branch. The medial branch joins the a. tibialis posterior superficialis and thus marks the point at which the adult a. tibialis posterior bifurcates into the aa. plantares.'' The absence of a normally developed a. plantaris medialis in these cases of absence of the a. tibialis posterior should therefore be expected, assuming that it is the terminal part of the a. tibialis posterior superficialis, which, apparently, failed in all of these cases to make its appearance.

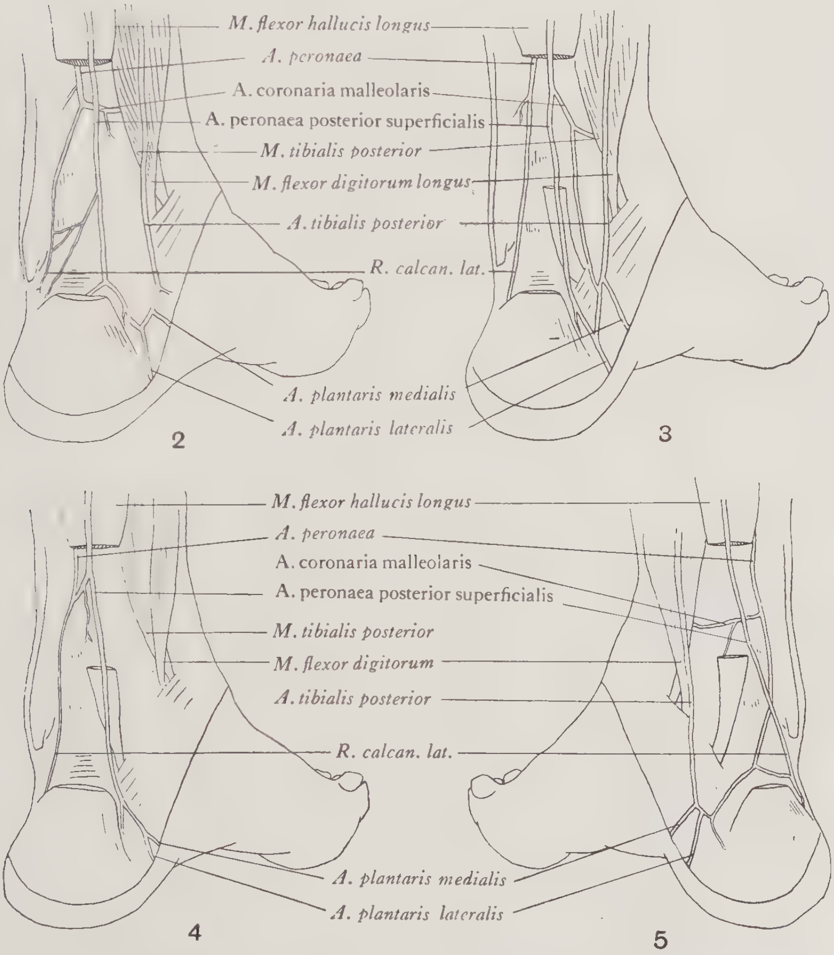
Yet in two of these cases there is a fully developed medial plantar artery, which, in the third case, is represented by a small vessel. It follows from this that the occurrence of the medial plantar artery is not necessarily dependent upon the presence in the embryo of the a. tibialis posterior superficialis. It seems probable, therefore, that the medial plantar artery is a channel through the medial part of the rete plantare, to which blood may be transmitted by any of the large arteries of the posterior crural region.

In three other limbs (figs. 2, 3, and 5) there are three large arteries in the posterior crural region:

1. The posterior tibial artery, which ends as the medial and lateral plantar arteries; it is somewhat smaller than usual.
2. A persisting a. peronaea posterior superficialis which furnishes, as usual, the first part of the peronaeal artery.
3. An imperfect peronaeal artery.

The persisting posterior superficial peronaeal artery, which appears to be a branch of the peronaeal, becomes free from that vessel at the junction of its first and second parts. It continues downward superficially to the m. flexor hallucis longus and ends by reinforcing the lateral plantar artery.

The lateral calcaneal branch, which arises from this embryonic artery, appears to have been taken over in all three


M. flexor hallucis longus
A. peronaea
A. coronaria malleolaris
A. peronaea posterior superficialis
M. tibialis posterior
M. flexor digitorum longus
A. tibialis posterior
R. calcan. lat.
A. plantaris medialis
A. plantaris lateralis
2
3
M. flexor hallucis longus
A. peronaea
A. coronaria malleolaris
A. peronaea posterior superficialis
M. tibialis posterior
M. flexor digitorum
A. tibialis posterior
R. calcan. lat.
A. plantaris medialis
A. plantaris lateralis
4
5

Figs. 2 to 5 The branches of the aa. peronaea posterior superficialis, peronaea and tibialis posterior. The embryonic posterior superficial personeal artery has persisted in its entirety and its lateral calcaneal branch has been taken over by the ramus communicans inferior. In figure 4 it communicates with the r. communicans inferior. Its relation in figure 2 is somewhat doubtful. The a. interossea is represented in figures 2 and 4.

of these limbs by the imperfect a. peronaea (figs. 2, 3, and 5). There can be no doubt of this in the limbs represented in figures 3 and 5, although some doubt remains as to the exact arrangement in that represented in figure 2. In this limb there is some question as to whether a direct calcaneal branch of the superficial posterior peronaeal is reinforced by a branch of the peronaeal or vice versa. The former seems to be the more probable alternative. In the limb represented in figure 4, in which there are only two arteries in the posterior crural region, the imperfect peronaeal artery certainly ends by reinforcing a lateral calcanaeal branch of a persisting superficial posterior peronaeal.

The main stem of the posterior superficial peronaeal artery ends by joining the lateral plantar a short distance beyond the terminal bifurcation of the posterior tibial.

The first part of the abnormal peronaeal artery is a derivative, as usual, of the embryonic superficial posterior peronaeal. The second part, also as usual, is the adult representative of the ramus communicans inferior. A part of the embryonic a. interossea does not appear to have formed the third part of the peronaeal artery as in normal cases. A part of the interosseous artery is recognizable in the limbs represented, respectively, in figures 2 and 5, but not in that represented in figure 3. The ramus communicans inferior appears, in fact, to have taken over the lateral calcaneal branch of the a. peronaea posterior superficialis, which usually forms nearly the whole of the fourth part of the peronaeal artery. In the limbs represented in figures 2 and 5 the interosseous appears to be a branch of the coronary artery, which is one of its branches in the embryo. The interosseous therefore does not form part of the peronaeal in either of these limbs or in that reproduced as figure 3. The third part of the ordinary peronaeal is thus absent in all of them, as it is in the limb reproduced as figure 4. In the cases of the figures 3 and 5 there was a ramus perforans arteriae peronaeae, but in that of figure 2 it was absent.

In the seventh limb of this series there are two large arteries in the posterior crural region, although the posterior tibial is absent (fig. 4). The plantar arteries, which are normally distributed, are the terminal branches of a persisting superficial posterior peronaeal artery. The first two parts of the peronaeal artery are normally formed, as in all the other limbs of this series. The part of the embryonic interosseous artery which usually forms the third part of the peronaeal is, it seems, represented in this case not as a part of that artery, but as a branch of the lateral calcaneal branch of the a. peronaea posterior superficialis. There is no vessel representing the coronary branch distinctly, but a large perforating branch forms a connection between the imperfect peronaeal artery and an unusually small a. tibialis anterior, which is reinforced by it. The second part of the peronaeal artery becomes continuous with the lateral calcaneal branch of the embryonic superficial posterior peronaeal, which forms the terminal (fourth part) of the normal peronaeal artery. The lateral calcaneal branch of the posterior superficial peronaeal has retained its original connection.

In this limb (as in two of those of type *a* represented in fig. 1) the medial plantar artery appears to be normally distributed. Further evidence is thus furnished that the formation of that artery is dependent upon the presence of a vessel capable of pouring blood into the medial region of the rete plantare irrespective of its nature.

Another unusual feature of this case is the large size of the branch of the medial inferior genicular a. This vessel, after having given off a branch distributed in the ordinary way, passes anteriorly to the m. popliteus and becomes continuous with the a. tibialis anterior. This channel is the persisting a. poplitea profunda.

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THE USE OF CEREBRAL ANEMIA IN EXPERIMENTAL EMBRYOLOGICAL STUDIES UPON MAMMALS

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The purpose of this study was to develop a method by which embryos of the rat can be exposed for study of their behavior without interference of anesthetics applied to the mother. The observations were made upon albino rats from the colony of the Department of Anatomy of the University of Kansas, where the work was done during the years 1921 to 1923. For financial aid in the work acknowledgment is due the Research Committee of the Graduate School of the University.

Bichat (1803), according to Minkowski ('22), was probably the first important observer of mammalian foetal movements. He made his observations on the foetal Guinea-pig. The writer has been unable to find out exactly what method he used.

Zuntz ('77) studied the antenatal movements of the Guinea-pig. The method which he used is briefly described as follows: The unanesthetized Guinea-pig was immersed in warm (38°C.) 0.6 per cent salt solution, with nose and mouth only above and out of the solution. The foetus was exposed, by means of an incision through the mother's abdominal wall and uterus, and examined without disturbing the placental circulation.

Later, Preyer ('85) made a detailed study of the foetal movements of the Guinea-pig. Even to-day his observations are of great importance. He employed several methods, evidently without the administration of anesthetics or narcotics, since in no case does he specifically state that they were used. The writer therefore assumes that Preyer's animals were unanesthetized. His methods may be briefly summarized as follows: 1) By inserting a long thin needle through the abdominal and uterine wall of a pregnant Guinea-pig and into the foetus, he was able to obtain distinct oscillations of the needle, caused by movements of the foetus. 2) With the aid of the stethoscope he detected sounds of foetal movements in mammals, especially during the later stages of pregnancy. 3) By means of incisions through the abdominal and uterine wall of the unanesthetized Guinea-pig or rabbit, the foetus was exposed and examined in physiological salt solution (0.6 per cent at 38°C.), either while connected to the mother's blood supply by the placenta and umbilical cord or while in the excised uterus. Most of Preyer's experiments were carried out by the latter method.

Graham Brown ('15) has studied the foetal movements of an 8 to 9 cm. cat. He describes his method very carefully as follows: "For the purposes of the experiment the cat (decerebrate) is laid upon the table beside a shallow vessel which contains warm physiological salt solution. The abdomen is slit and a loop of uterus brought out. From this the foetus is quickly shelled out into the dish of saline solution—care being taken to leave the umbilical cord intact and the foetal circulation unimpeded. The foetuses carefully examined were four in number."

Lane ('17) observed the activities of foetal albino rats. Three stages were carefully investigated by Lane—the 7½-mm. embryo, the 16-mm., and the 22-mm. foetus. The pregnant rat was killed by quickly severing the cervical cord with a bone forceps. The uterus was excised and the foetuses examined in a warm chamber (38°) while covered with a modified Ringer's solution.

Minkowski ('22) examined twenty human foetuses obtained as a result of Caesarean operations. The foetuses were removed while the mother was under local anesthetic (novocaine adrenalin 60 to 90 cc. 0.5 per cent solution) and examined in a bath containing warm physiological salt solution. All except two of these foetuses suffered from asphyxia, as evidenced by the degree of cyanosis.

Of the methods cited above, all but that devised by Graham Brown ('15) seem to the writer objectionable as a means to trustworthy results. Operations without anesthesia are neither desirable nor permissible, while excision of the uterus destroys the normal relation to the maternal blood. Brown's method obviates the above objection, but it involves the difficulties of decerebration. To avoid this surgical procedure the writer uses cerebral anemia, which he induces by the following method.

The pregnant female rat is anesthetized with ether under an inverted 6-inch funnel. When the animal is sufficiently under the influence of the ether it is quickly and securely tied by all four feet, back down, to a heavy wire frame. This frame is made to fit into the water-bath, which is described below, where it rests when in place on two longitudinal shelves, 4 inches from the bottom of the bath. The neck of the rat is extended by means of a strong thread, tied to its two upper front teeth and the edge of the screen. Ether is now administered by the drop method, a piece of cotton serving the purpose of the mask. With a sharp scalpel an incision is made through the skin of the neck in the median line from a point a little cephalad of the angle of the jaw to the sternum. The large glands of the neck, which cover the trachea and sternocleidomastoid muscles, are carefully dissected free from their fascial coverings and turned laterally. The trachea and sternocleidomastoid muscle now come into view. With two very smooth seekers made of bone and sharpened to a point, the carotid arteries are secured and each in turn carefully separated from the accompanying vagosympathetic nerve and ligated as close to their origin as possible. The large right and left external jugular veins are secured and ligated close to where they empty into the innominate veins. Ether is now discontinued, the skin incision quickly closed with a few stitches, and the rat, made fast to the rack as described above, is then transferred to the water-bath, where it is immersed in warm (38°C.) physiological salt solution, care being taken to keep the nose and mouth above and out of the solution.

When the temperature of the water is again constant at 38°C., the abdomen is opened with a scalpel in the midline. The incision is made through the linea alba in order to avoid hemorrhage. The right and left horns of the uterus are secured and lifted out of the peritoneal cavity. The other viscera are prevented from protruding through the incision by gently packing against them small loose balls of absorbent cotton. Finally, through incisions made in the pale and clear part of the uterine wall, the foetuses are one by one exposed. If the temperature of the bath is constant and at 38°C., the amnion may be opened and the foetus safely shelled out.

Great care must be exercised in handling the foetus. It should rest on some absorbent cotton close to the placenta so as to prevent the stretching of the umbilical cord. This done, electrical and tactile stimuli may be applied to the foetus, and the results noted. By this method living foetuses of the rat have been under observation continuously for two hours.

From the behavior of male rats with jugular veins and carotid arteries ligated as here described the writer is convinced that the condition produced by the ligation is essentially that of decerebration.

The water-bath used in these experiments was originally designed for serological work by the Electric Sales Company, Berkeley, California. The size of the bath proper is 7 × 8 × 12 inches. The specially designed thermostat insures constant temperature of 38°C. The apparatus is indeed conveniently designed and meets the requirement splendidly.

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PERFORATION OF THE SEPTUM PELLUCIDUM OF THE BRAIN

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ONE FIGURE

In June, 1924, Gibson placed on record in this journal a case of perforation of the septum pellucidum, and stated that he had been unable to find an account of a similar case.

That his case of this condition is at least not unique is evident from the accompanying drawing of a specimen I removed some years ago from the brain of a dissecting-room subject and placed in the museum of my department on account of such an abnormality.

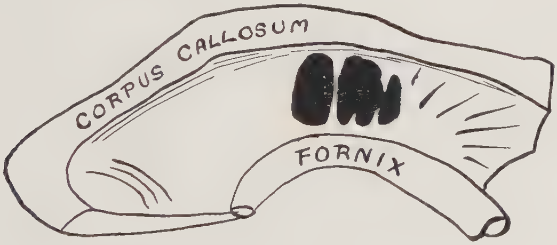

Figure 1

The portions of brain preserved are the septum pellucidum and the parts of the corpus callosum and fornix which encircle it. Toward the posterior end of the septum two large apertures, separated from each other by a thread of tissue, may be seen.

As in Gibson's case, the subject was an elderly man, whose lateral ventricles were enlarged. In fact, a note made at the time describes them as being 'hydrocephalic.' As incidental observations may be mentioned the facts that the upper and lower jaws were edentulous, that the posterior nasal spine was notched or cleft in the middle line (though there was no cleft of the soft palate), and that the ethmoidal, maxillary, and mastoid air cells were so remarkably thin-walled and extensive that they likewise were preserved as museum specimens.

The condition was regarded as an acquired one: an atrophy secondary to the ventricular distention, and as analogous in a sense to the manner in which the foramina of Magendie and Luschka were formed in earlier life.

A second case, one with even larger perforations, has now come under my notice. It is in a formalin-hardened adult brain, which, like the first case, had marked distention of the lateral ventricles.

These cases substantiate that of Gibson and are perhaps not without practical interest.

SUR L'HISTOPHYSIOLOGIE DE L'HYPOPHYSE HUMAINE

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DOUZE FIGURES

Dans l'état normal, l'activité endocrine de l'hypophyse s'exerce par des moyens histologiques differents et nous devons distinguer les processus de sécretion dans la partie médullaire et dans la partie corticale du lobe antérieur de l'hypophyse et dans la partie intermediaire et nous pouvons croire aussi à la sécrétion supposée du lobe nerveux.

Dans la partie médullaire du lobe antérieur on trouve des signes abondants et encombrants de l'activité granuleuse siderophile (méthode Heidenhain) et ses processus excrétoires. Dans la partie corticale du lobe grandulaire ou lobe antérieur on trouve la plus grande partie des cellules avec l'aspect éosinophile et entre elles apparaissent d'abondantes masses siderophiles emprisonées dans des espaces dont les parois sont formés de cellules qui ont la même éosinophilie mais d'aspects assez differents dans la forme, dans leur état, grandeur et indices d'activité antérieure.

Dans la partie médullaire comme le montre la fig. 1 on voit les processus de sécrétion que j'ai déjà décrit dans le chien (L'hypophyse à l'état normal, Annais Scientificos da Faculdade de Medecina do Porto, vol. III, No. 4, et vol. IV, No. I): les grains de sécrétion peuvent se jetter directement dans les vaisseaux sanguins que dans certains points de l'hypophyse sont plutôt des tuyaux creusés entre les cordons glandulaires comme l'on voit en b fig. 1; d'autres fois les grains de sécrétion sont excrétés dans un espace creusé entre les cellules, ils se fondent en une masse foncée siderophile laquelle revient peu á peu comme l'on voit en c fig. 1.

Dans la même figure l'on voit des cellules completement épuisées ou en dégénérescence. Les divers processus de sécrétion hypophysaire se trouvent dans la partie médullaire de l'hypophyse de l'homme dans ces zones differentes et limitées montrant nettement comment une glande endocrine peut recourir simultanement à ses processus de travail histologiques.

Que ces masses siderophiles de la partie médullaire du lobe antérieur sont le resultat de la fusion des grains, c'est bien evident si nous comparons la fig. 8 de mon travail (L'hypophyse à l'état normal) avec la presente fig. 1.

Dans la première on voit les grains de sécrétion encore séparés dans un pseudo-follicule du lobe glandulaire de l'hypophyse du chien.

Les figures 2 et 3 montrent des masses siderophiles de la partie médullaire, apparues par un tel processus entourées de cellules dans des differents états d'activité.

Si ce produit colloide peut arriver dans un vaisseau par un chemin taillé par la dégénérescence d'une cellule en couloir, il s'écoule dans le courant sanguin et il reste un nouvel intervale destiné à recevoir de nouveaux produits de sécrétion. Si la colloide ne peut pas atteindre ce chemin, les cellules devront verser leurs produits dans des espaces créés à nouveau.

Pour la commodité de description j'appelle colloide tout produit qui se trouve dans ces cavitées formées par écartement des cellules qu'elles aient la propriété siderophile ou éosinophile. La colloide que ne puisse pas s'écouler dans les vaisseaux tant qu'elle sera dans les espaces intercellulaires subit ce que j'apelle la regréssion siderophile.

Cette regression siderophile se manifeste par ce que la masse colloide noire, qu'a fixé la laque ferrique en fig. 3, perd peu à peu sa propriété éosinophile.

Dans ce cas il commence à apparaitre un cercle avec la couleur d'éosine contournant la masse centrale foncée. Ce

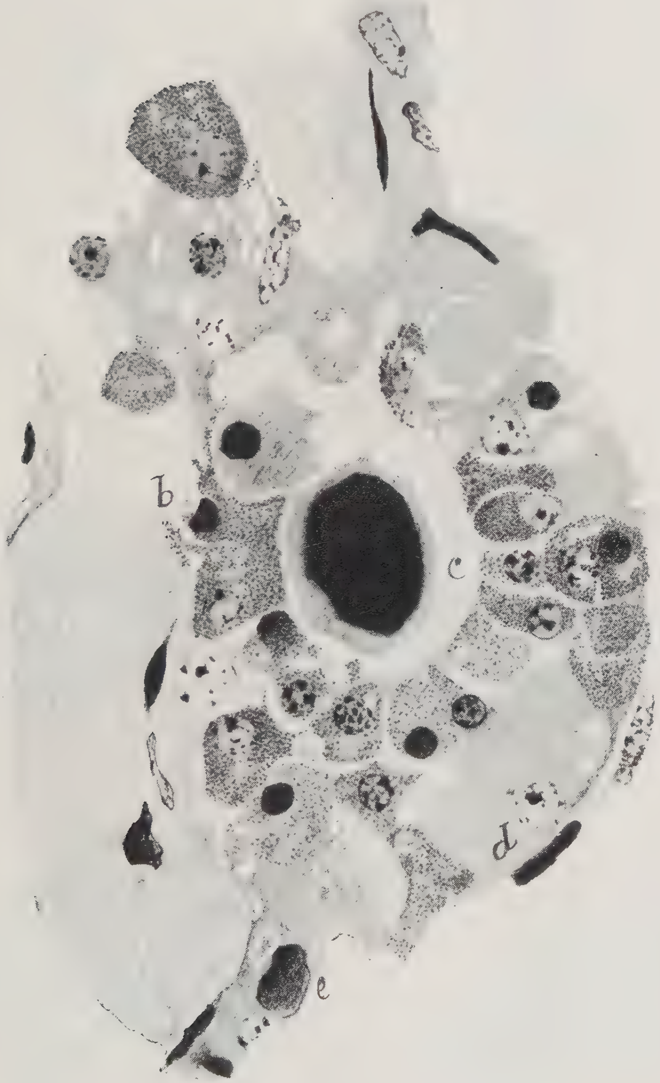


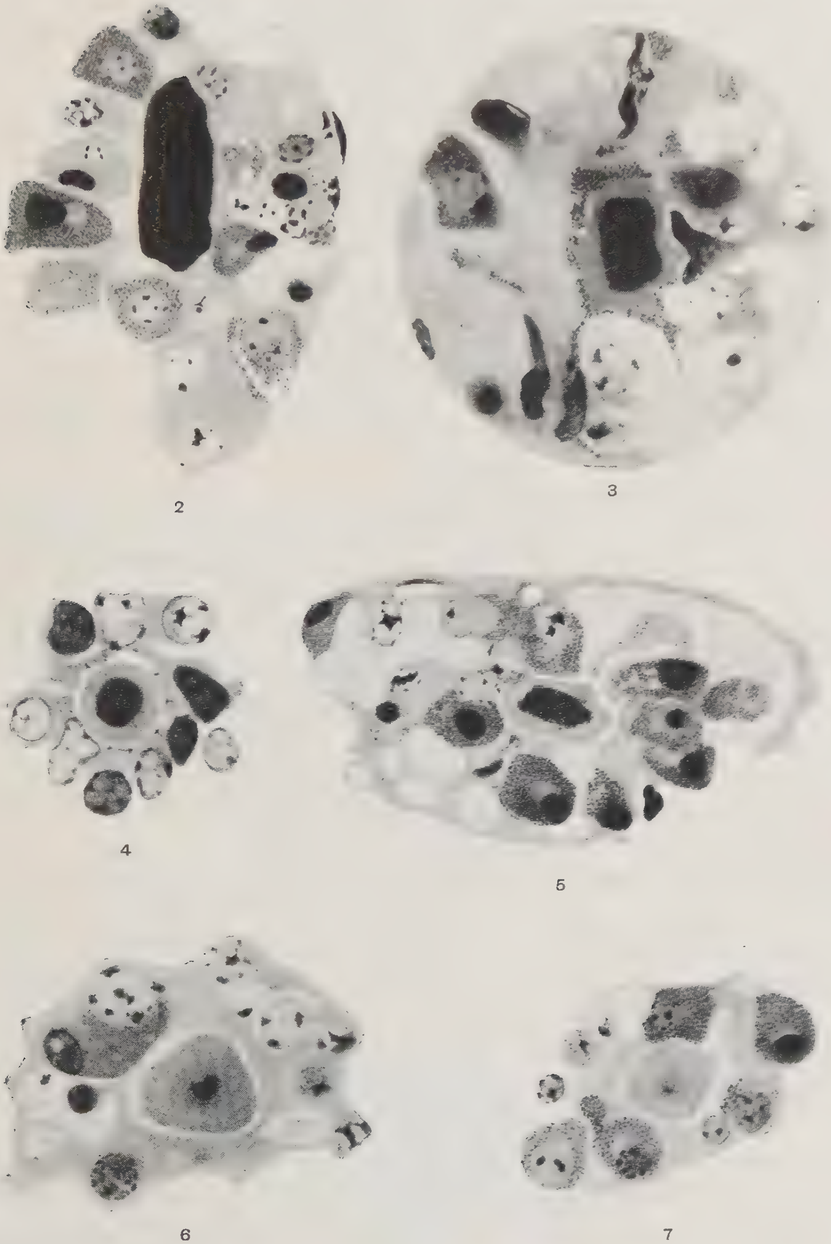
Fig. 1

cercle va en augmentant et envahissant la masse centrale foncée de la péripherie vers le centre tandis que celle-ci revient peu à peu et en diminuent au fur et à mesure de l'invasion éosinophile.

Les figures 1 et 3 répresent les phases de ce phénomène. Pour confirmer ce phénomène j'ai eu recours à l'hypophyse de l'enfant de 7 ans où la régréssion présente des étapes et des nuances lentes et avec toutes les phases extremes et intermediaires.

Si les figures representaient des dégénérescences cellulaires on ne comprendrait pas comment la partie centrale de ces figures devraient representer toujours en reduction la forme précise de l'espace rempli de colloide. Et encore davantage, comment la masse centrale siderophile, plus dense, n'était pas poussé par l'action de la presenteur, vers les points de plus grande déclive et restait toujours centrale. Si l'espace est circulaire, elliptique, triangulaire etc, la partie centrale siderophile s'adapte toujours à la forme de l'espace contenant ayant néamoins une plus grande tendance vers la forme de lignes circulaires que vers les lignes anguleuses.

Ce fait ne se voit plus dans la partie corticale du lobe antérieur de l'hypophyse oú la masse siderophile n'occupe pas la partie centrale. Nous devons croire que ces masses sont produits d'excretion normale dans la portion médullaire du lobe antérieur du corps pituitaire et qu'ils constituent une des différentes formes par laquelle se manifeste l'activité hypophysaire. Les fig. 4 et 5 montrent deux pseudo-follicules avec des produits de sécrétion dans des phases proches de la régréssion siderophile. La fig. 6 montre un espace triangulaire avec la partie centrale colloide dans un dégré avancé de régréssion siderophile, celle-ci étant cernée par une atmosphere poussièreuse qui n'a pas été completement liquifiée par la régression. La fig. 7 montre la colloide avec son aspect éosinophile avec une nebuleuse très tenue. C'est pour ainsi dire une des étapes finales de la régréssion. Dés ce moment les espaces apparaissent pleins de colloide avec son aspect nettement éosinophile parfaitement adaptée aux



Figs. 2–7

intervalles oú elle est contenue. Toutes les phases de régréssion representées par les figures 4, 5, 6, et 7 ont lieu dans l'hypophyse de l'enfant.

Dans la partie corticale du lobe antérieur apparait le processus de dégénérescence comme une variation du processus de sécrétion. Les cellules de la portion corticale du lobe antérieur (après que son protoplasme aura accumulé des produits de sécrétion) dégénérent et cette dégénérescence invahit des cordons entiers et les produits resultants de la dégénérescence cellulaire forment de veritables masses adaptées aux tuyaux dont les parois sont formées par les cellules qui n'ont pas dégénéré et qui encercle les autres. Ce sont ces masses et ces tubes donnant à la coupe les aspects des fig. 8, 9, 10, 11, et 12.

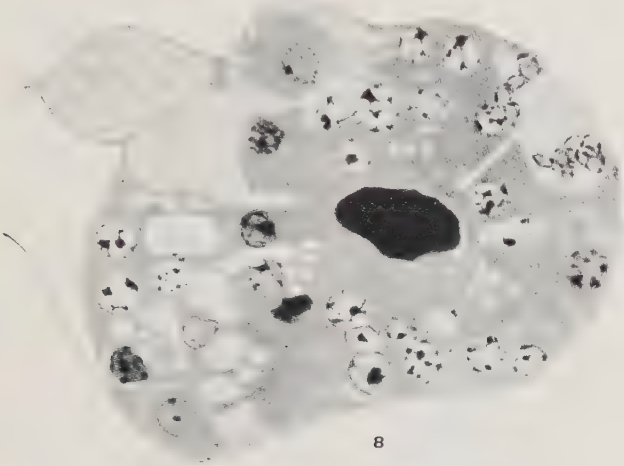
Quand la dégénérescence cellulaire a lieu les cellules restent dans un état semiliquide, colloide, dans lesquelles, il y a deux portions d'une densité inégale, le noyau et le protoplasme, le premier plus dense, se déplace peu à peu à l'interieur de la cellule dégénérée et se rapproche du noyau des autres cellules.

Il se rapprochent par affinité de densité et d'état physique ainsi comme les protoplasmes se fondent par le même motif, ces derniers donnant lieu aux masses éosinophiles qui entourent la partie centrale siderophile. Cette dernière est très volumineuse parce que les noyaux dégénérés prennent un grand volume comparé à celui de la cellule.

La dégénérescence n'a pas toujours lieu en masse, elle n'affecte pas non plus toujours les cordons entiers.

On voit dans la partie corticale des cellules isolées et dans divers états de dégénérescence. Pour les processus de dégénérescence dans la partie corticale de l'hypophyse la régréssion siderophile ne se fait qu'avec la partie centrale siderophile provenant de la fusion nucléaire.

Certainment il y a quelque relation entre les deux processus de régréssion de la partie corticale et de la partie médullaire dans la partie qui touche à la future substance qui doit entrer dans la voie circulatoire. La différence qui existe dans la régréssion siderophile des produits des deux parties c'est



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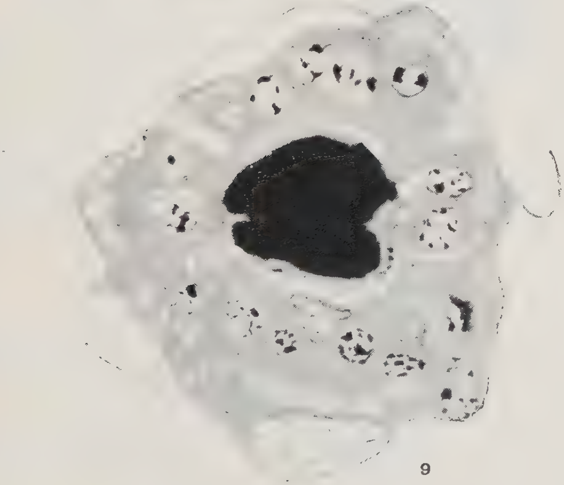

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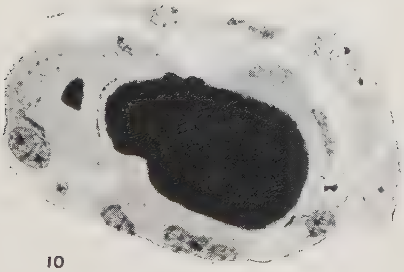

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Figs. 8–10

que dans la partie corticale il y a au début un cercle éosinophile appartenant à l'ancienne partie protoplasmatique dégénérée, ce cercle n'existant pas dans les primitives masses siderophiles de la portion médullaire, parceque les produits primitifs siderophiles de sécrétion (fig. 2) resultent seulement de la fusion des grains, tandis que dans la portion corticale il faut toujours considerer (fig. 8, 9, 10, 11, et 12) la partie centrale siderophile de la fusion des noyaux et la partie peripherique éosinophile de la fusion des protoplasmes.

Que represente cette régréssion dans l'histophysiologie de l'hypophyse?

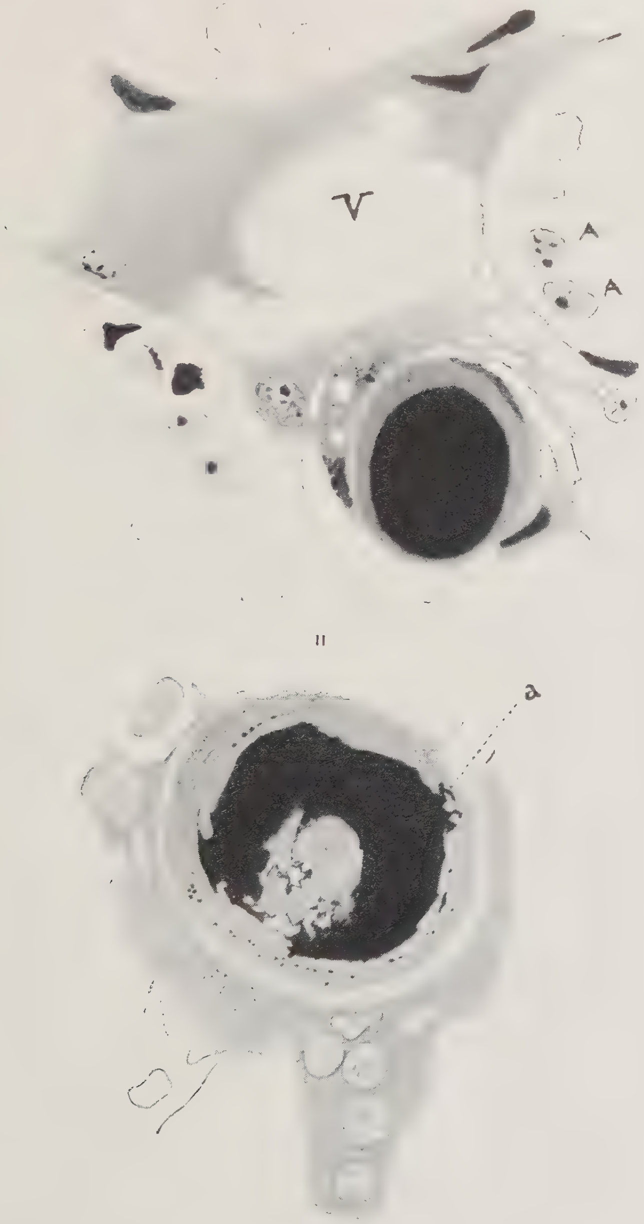
Certainement cette explication nous est encore inconnue et nous ne pouvons pas trouver le mystère du compliqué travail histologique de l'hypophyse. Nous pouvons seulement pour le moment rester avec une vague notion qui nous satisfait provisoirement de ce que la regression siderophile represente peut-être des phases d'une transformation albuminoide, que c'est une manière d'emprisoner dans ce nouveau albuminoide des hormones qui serviront dans une periode plus ou moins longue et qui sont des reserves pour la defense commune que nous offrent les glandes à sécrétion interne.

RÉSUMÉ

O corpo pituitario revela trabalhos histologicos differentes na parte cortical e na parte medullar do lobo anterior embora os productos elaborados tenham egual destino.

Na parte medullar os productos de secreção resultam da fusão dos grãos siderophilos das cellulas e que se dá nos espaços intercellulares.

Na parte cortical as massas siderophilas sao productos da degenerescençia cellular. N'uma e n'outra ha a regressão siderophila da colloide: na parte medullar a regressão é completa e a colloide siderophila torna-se éosinophila; na parte cortical, como os productos colloides, resultantes da degenerescencia, são éosinophilos no perifera (que corresponde ao protoplasma degenerado) e são siderophilos no centro (que corresponde aos nucleos degenerados que se fundiram) a regressão só se dá na parte siderophila.



11

12

Figs. 11–12

BOOKS AND PUBLICATIONS

PREPARATION OF SCIENTIFIC AND TECHNICAL PAPERS, by Sam F. Trelease and Emma Sarepta Yule; 1925, 114 pages. Size 5 × 7¼, buckram, $1.50, Williams & Wilkins Company, Baltimore. This manual is intended for use of students who are writing articles on scientific or technical subjects. It is designed primarily as a reference book for senior and graduate students who want information concerning matters of style to be followed in the preparation of papers. It is not a collection of theories; it grew from suggestions first used in mimeographed form and adopted by the College of Agriculture (Philippines), being used as a supplementary text book in teaching theme writing in the English courses. The directions apply to the writing of reports in science, agriculture, engineering, and medicine, and to the preparing of manuscripts of a more popular nature on scientific or technical subjects. *An abstract from the Preface.* This book in size, contents, arrangement and purpose is admirable, and will have a far wider range of usefulness than to merely serve the needs of senior and graduate students.

THE AUTHORS' BOOK: Being notes for the guidance of authors in dealing with publishers, preparing manuscripts, and reading proofs; 1925, 74 pages, size 6 × 9, paper cover. The Macmillan Company, New York. This is a new and revised edition of a little book previously published under the title Notes for the Guidance of Authors. It includes directions for preparing and submitting manuscripts, for proofreading, with notes on style, legal considerations, promotion, and contains a glossary of terms.

METHODS AND PROBLEMS OF MEDICAL EDUCATION. 1911 (Second Series), 1925. 118 pages, 26 illustrations, size 8 × 11. Division of Medical Education, The Rockefeller Foundation, New York. *Prefatory Note.* In the present period of unprecedented progress and readjustment in medicine, the problem of dissemination of information is becoming increasingly difficult and perplexing. To avoid some of the difficulties, it has seemed advisable to collect and publish from time to time brief description of clinics, laboratories, and methods of teaching in different parts of the world in order that the information in convenient form may be brought to the attention of those most interested. It is hoped that the material may be of assistance to those planning improvements in building and methods. The collection of articles here presented constitute the initial effort in this venture.

PLASTISCHE ANATOMIE, Die konstruktive Form des menschlichen Körpers, von S. Mollier, mit Bildern von Hermann Sachs, 1924; 296 pages, 464 illustrations (many of them in colors). Size 9 × 11½, cloth (stiff cover), $12.85. Verlag von J. F. Bergmann, München. The illustrations are exceptionally well handled and instructive.

MEMORIAS DO HOSPICIO DE JUQUERY, Sao Paulo (Brasil). Anno I–Num. 1, 1924; 346 pages, 6½ × 10, illustrated, paper cover, containing an introduction and thirteen papers by six authors.

BOOKS AND PUBLICATIONS

MEDICAL SCIENCE, Abstracts and Reviews, volume 12, number 2, May, 1925. Published for The Medical Research Council by Humphrey Milford, Oxford University Press, England; 6 × 9¾, pages 91 to 176, 3 shillings per month, 30 shillings per year. Contents. The treatment of acute appendicitis; Abstracts, general medicine, cardio-vascular diseases, respiratory diseases, metabolic diseases, neurology, surgery, pathology, bacteriology, radiology.

MEMORIAS DEL INSTITUTO ESPAÑOL DE OCEANOGRAFIA, Publicadas bajo la dirección del Pr. Odón de Buen. (10¾ × 13¾) Tomo II, Memoria II, Trabajos realizados en los años de 1916, 1917 y 1918 por las costas de Galicia, Asturias y Santander. Investigaciones Quimicas por D. Antonio Ipiens Lacasa. Madrid, 1919, 78 pages.

Tomo III, Memoria 1a. Investigaciones sobre el tejido cartilaginoso de los selacios por Manuel Sánchez y Sánchez, Madrid, 1920. 32 pages.

Tomo III, Memoria 2a. Recherches anatomiques sur l'appareil génito-urinaire mâle du Mesoplodon et des cétacés en général par R. Anthony. Madrid, 1922. 126 pages.

Tomo III, Memoria 3a. Gobius de la Peninsula Ibérica y Baleares, Grupos Lesueurii, Colonianus, Affinis y Minutus, por Fernando de Buen. Madrid, 1923. 146 pages.

Tomo III, Memoria III. Relaciones hidrográficas y agua normal. Estudio critico y experimental, por José Giral y Pereira. Madrid, 1924. 54 pages.

A new international scientific journal has recently appeared in Germany—the "Archiv für experimentelle Zellforschung, besonders Gewebszüchtung (Explantation)" (Archives for experimental cytology, especially devoted to tissue cultures (explantation)). The publisher is Gustav Fischer in Jena. The Archives are open to the scientists of all nations and the papers can be published in German, English, French, or Italian. Each paper should be accompanied by an abstract. In addition to original papers, short critical reviews shall also be admitted. Special care will be taken for the adequate reproduction of the necessary plates. The issue of the numbers, each comprising about 160 pages, shall be independent of the calendar year and the papers will be published within the shortest time possible. The first number is just out; its price is 8 Goldmarks (about $2.00). The managing editor is Prof. Rhoda Erdmann, in Berlin. The Associate Editors are: Aubrey Drew, London; Montrose Burrows, St. Louis; Albert Fischer, Kopenhagen; A. Krontowski, Kiew; Ernst Küster, Giessen; Giuseppe Levi, Torin; Margaret Lewis, Baltimore; Warren Lewis, Baltimore; Leo Loeb, St. Louis, Alexander Maximow, Chicago; R. Rössle, Basel.

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THE MORPHOLOGY OF THE VASCULAR SYSTEM OF THE RICHARDSON GROUND-SQUIRREL, CITELLUS RICHARDSONII, SABINE

GEORGE F. SLEGGS
University of Saskatchewan

TEN PLATES

The ground-squirrel, Citellus richardsonii[1] (Sabine), commonly known as the gopher,[2] is the almost ubiquitous burrowing mammal of the prairie in Manitoba, Saskatchewan, and Alberta.

Some fifty species of the genus Citellus are recognized in the United States National Museum Bulletin no. 128.[3] These ground-squirrels are widely distributed over North America, and their convenient size and the ease with which they may be obtained by trapping would seem to render them well adapted to class use as a mammalian type, especially where the cost of obtaining preserved cat or rabbit material from a distant supply house is a consideration.

Since the vascular system usually presents the bulk of a mammalian dissection and is that portion of the work in which the student requires most detailed guidance, the author hopes that this paper may be welcomed by some for that reason if for no other.

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[1] Syn. Arctomys richardsonii, Sabine ('22); Spermophilus richardsoni richardsoni, True ('85). Family Sciuridae, subfamily Sciurinae.

[2] The true gopher, or pocket gopher, Geomys bursarius, belongs to the family Geomyidae, differing from the Sciuridae which includes the burrowing squirrels. The burrows of the true gopher, unlike those of the ground squirrel, have no exit above ground, the animal only coming to the surface to throw out the excavated earth.

[3] List of North American recent mammals, by Gerrit S. Miller, Jr.

THE ANATOMICAL RECORD, VOL. 30, NO. 3
JUNE, 1925

In the account which follows attention is directed particularly to those features of the vascular system which are commonly subject to modification in mammalian anatomy. Structures which are typical, such as the heart, are not described, nor does space permit of a treatment of the variations in the arrangements of the peripheral vessels and the arteries and veins of the extremities, which presumably arise out of the small size of the animal. The larger trunks, however, are definitely established, and it is hoped that this paper presents a description and discussion of a number of features of importance from the point of view of comparative anatomy. These are listed in the summary. In cases where existing nomenclature is inadequate or inappropriate, new nomenclature is suggested, provisionally, for some of the main vessels. In the case of the minor vessels, such a procedure would be too cumbrous, and they are accordingly enumerated by indicating the parts which they supply.

The dissections were performed several times, in the case of the arteries by the aid of gelatin-chrome-yellow injection, either forward or backward through the aorta.

The vascular system will now be described seriatim, special features in the general anatomy being referred to from time to time in connection with the modifications of the vessels.

THE AORTIC ARCH AND THE BRANCHES OF THE COMMON CAROTID ARTERY (fig. 1)

From the arch of the aorta (*1*) spring two large arteries: the brachiocephalic (*2*), which runs forward slightly to the right of the midline, and the left subclavian (*3*), which runs out to the left, arising from the aortic arch beyond the brachiocephalic.

In all of the specimens examined, the left common carotid (*5*) came off from the brachiocephalic (as in the cat rather than the rabbit, where the left common carotid usually comes off independently from the aortic arch).

The right subclavian (*7*) is given off one-third of an inch or so beyond the origin of the left common carotid. The right common carotid (*6*) proceeds forward to the head region, slightly diverging from the trachea and passing out of view dorsally over the sternohyoid muscle (fig. 2, *27*).

The branches of the right common carotid (or of the left which are similar) are as follows.

At the level of the thyroid gland the internal carotid (*9*) comes off dorsally and runs to the tympanic bone of the skull, through which it enters the cranial cavity.

At the same level branches are given off to the thyroid (*11*) and to the occipital region (*10*).

The main trunk, now the external carotid (*17*), passes dorsally over the ligament of the digastric muscle and supplies the larynx by a branch (*12*).

The external carotid, just in front of the larynx and medial to the angle of the jaws, then breaks up into three extensive branches.

The first of these (*15*) runs along the medial side of the masseter and gives off the lingual artery (*26*), which runs in the tongue, giving off numerous side branches into the tongue muscle. The vessel *15* continues straight on to supply the anterior portion of the floor of the mouth (*25*), and it also supplies the sublingual gland by several small branches (*27*).

The second main branch of the external carotid (*14*) runs slightly laterally and bifurcates to supply the masseter (by *24*) and the submaxillary gland (by *23*).

The third branch (*13*) bends sharply laterally around the posterior margin of the masseter. It supplies the parotid (by *18*), a lymph node (by *19*), and then runs to the angle of the jaw, to which it gives off a branch (*29*). The main stem turns in medially and passes internal to the masseter, eventually supplying the pharynx (by *21*) and the root of the tongue (by *22*) and adjacent hyoid region.

With regard to the nomenclature of these vessels, it may be pointed out that they differ considerably in their arrangement from the branches of the carotid in the cat and the rabbit or man. The third branch (*13* and *16*) may be fairly safely termed the external maxillary, while its portion beyond (*29*) would correspond to the internal maxillary.

The pinna is greatly reduced, so that the auricular branches are recognizable with difficulty. Small branches from the scalp and ear open in some cases into the branch to the parotid, in other cases directly into the external maxillary. Frequently the parotid is supplied by additional small branches from the adjacent branches of the external maxillary (*19, 29*).

THE SUBCLAVIAN ARTERY (fig. 2)

The subclavian artery arises, on the left (*26*), directly from the arch of the aorta (*25*), and on the right (*32*) from the brachiocephalic (*33*). They are invested at their base by the thymus. The subclavian gives off posteriorly and ventrally the internal mammary artery (*11*), which runs ventrally along the inner side of the chest wall about an eighth of an inch to the side of the sternebrae, so that the right and left internal mammaries are here separated by a distance of a quarter of an inch or so. They communicate by anastomoses of small branches running across the sternum and by a definite transverse connection running close behind the posterior border of the ensiform cartilage (fig. 6 illustrates this connection in the case of the veins, whose course is similar.)

The internal mammary gives off branches which run laterally and dorsally in the chest wall along the posterior borders of the ribs. These branches anastomose at *X,X,X,X* with the branches of the supreme intercostal (*12*), in the case of the first three ribs, or with the intercostals that arise directly from the aorta (*17*) to supply the intercostal muscles posterior to the fourth rib.

The supreme intercostal (*12*) arises in some cases from the internal mammary near its base or from the subclavian directly. It supplies the intercostal muscle behind the first three ribs by the branches indicated by the anastomosis at *X*.

The remaining intercostal muscles are supplied directly from the aorta by the intercostal vessels, the first of which (*17*) is illustrated near the bottom edge of the figure. The anastomoses which take place at the junction of vertebral and sternal portions of the ribs have already been referred to.

Returning to the supreme intercostal, it was found that in one specimen under dissection this vessel gave off a very delicate branch (*14*), which pursued a tortuous course against the mediastinal peritoneum to end in the oesophagus (*16*) and giving off branches to the thymus (*13*) and a number of small vessels to the lungs (*15*). The latter are evidently the bronchials, and while their association with the supreme intercostal might be expected, their more direct relationship to the oesophageal is somewhat irregular.

In other specimens, even with an injection so penetrating that the arteries were colored to the limit of visibility, no bronchials were found. The absence, or apparent absence, of the bronchials is, however, not to be wondered at, since in the cat, which is considerably larger, the bronchials are often reduced almost to disappearance. The vessel *14* also gave off a small artery (*30*) to the mediastinum itself.

The blood supply to the thymus is variable; the artery supplying it on each side sometimes arising more proximally, from the subclavian of the brachiocephalic. In other cases several small vessels ran to the thymus.

The vertebral artery (*31*) leaves the subclavian just before it emerges from the chest cavity. It runs dorsally and medially and slightly forward to enter the vertebrarterial canal of the neck in company with the vertebral vein.

The subclavian now runs outward from the thorax over the first rib pressing against it on its medial side. Continuing as the axillary (*34*), it runs between two prominent nerves of the brachial plexus (*4*). The axillary gives off first a vessel marked *5,* which runs forward and laterally to the region of the venous circle upon the precaval vein (fig. 5, p. 177). It is significant that this artery itself describes a sharp curve here, accompanying the venous circle over a portion of its course, closely apposed to it, and giving off branches to the adjacent muscles and lymph nodes. There is, however, no double passage for the arterial blood, as in the case of the veins (fig. 5).

The axillary artery gives off a branch (*3*) which proceeds dorsally between the muscles of the upper arm, sending off a vessel (*2*) to the ventral side of the head of the humerus and then on to the shoulder. *3* is presumably the subscapular.

The external mammary artery (*9*), which leaves the axillary, is an extensive vessel running ventrally in the subcutaneous tissue outside the chest wall. It supplies in the female the mammary gland (*19*) by branches marked (*29*), and ramifies under the skin, anastomosing posteriorly with the superficial epigastric and the 'lateral cutaneous' (fig. 3, p. 174).

A branch (*10*) given off by the external mammary runs to the pectoralis major muscle (the ventral thoracic artery).

1 runs to one of the axillary lymph nodes.

8 is the thoracicodorsal artery, supplying the latissimus dorsi muscle.

7 supplies the flexor muscles of the upper arm.

The remaining branches of the brachial to the muscles of the arm are variable, and while figure 2 illustrates their disposition in a particular specimen, their further enumeration is not attempted.

THE BRANCHES OF THE ABDOMINAL AORTA (fig. 3)

The unpaired visceral branches of the aorta are specially described in the next section with reference to the gut, the liver, pancreas, and spleen. The coeliac artery (*9*) is given off almost immediately after the aorta has traversed the diaphragm. The anterior mesenteric artery (*10*) comes off sometimes very close (one-eighth of an inch) behind the coeliac, in some cases farther back. Between the coeliac and the anterior mesenteric the right renal artery (*16*) passes out to the right kidney, bifurcating just before it enters the hilus of the kidney and giving off from the region of the fork a pair of small arteries (*6* and *17*), one of which (*17*) runs in the fat applied to the posterior medial surface of the kidney, the other (*6*) also running in the fat situated anteriorly, but supplying in addition the right adrenal gland (*5*).

From the right renal artery a branch (*13*) arises which runs outward and backward along the dorsal body wall, dividing into three branches (*20*, *22*, and *23*). The presence of these three vessels is very characteristic. They supply the epaxial muscles of the back and the dorsal portion of the abdominal wall and they may penetrate the body wall to supply by small branches the subcutaneous tissue. In the specimen from which figure 3 was drawn, *20* gave off a branch (*18*) to the sacrospinalis muscle and then continued half way down the abdominal wall (*19*), which it supplied, and then passed out into the panniculus adiposus.

The second branch of *13* (viz., *22*) pursued a course parallel to *19,* while the third (*23*) divided into two, supplying the sacrospinalis posteriorly.

Passing now over to the left side, we again find three corresponding vessels (*12, 25,* and *26*), though their relationships to the central trunks are different; asymmetry, indeed, being a constant feature. The first one (*12*) of the three arises independently from the aorta, while the second and third (*25* and *26*) arise by a common stem (*24*) which originates close to the left renal artery.

For these three vessels the term 'lateral lumbars' is suggested and will be used in this paper. The evidence of their distribution indicates that they are derived, either wholly or partially, from the adrenolumbars of the cat or rabbit, which have undergone subdivision and migration from a position anterior to the renal arteries to a position either upon the posterior wall of the renal arteries or the aorta still farther back. Figure 7, for the corresponding veins, which accompany the arteries, exhibits a variation from the condition shown in figure 3, but here again the relationship to the central trunk, as explained, is apparent (in another specimen).

The left renal artery (*16*) is longer than the right and tends to bifurcate earlier, and in some cases is double from its commencement. It gives off, like the right, an anterior branch (*6*) which supplies the fat around the kidney in front of the hilus and supplies the left adrenal gland (*5*). A similar small posterior branch (*17*) supplies the posterior fat of the kidney, and this was found to give off, in several cases, an offshoot (*27*) which ran back to the left ovary. This offshoot is referred to later on, since the ovarian artery frequently comes off, in mammalian anatomy, from the renal artery on the left side.

A pair of lumbar arteries (*11*) leave the aorta at the level of the left kidney, and for a distance of about an inch the aorta continues devoid of branches other than two more pairs of small lumbar arteries. On a level with the fourth pair of lumbars, a pair of vessels (*15, 15*) arise laterally, and run out into the fat-distended broad ligaments of the uterus (fig. 10). Each of these gives off an anterior branch (*28*) into the broad ligament (which extends forward considerably) (fig. 10), and on the left this last-named branch anastomoses with the offshoot (*27*), previously referred to as coming from the left renal artery. The vessels *15* branch extensively in the uterine ligament, supplying the ovaries themselves, and anastomosing with the forward branches of the uterine artery (*35*). They are termed here the ovarian arteries, although the occurrence of a connection (*27*) from the left renal to the ovary by anastomosis furnishes a very common relationship of a true gonadial vessel, and might suggest that *27* is the true ovarian, greatly reduced, and which has been replaced by the larger vessel (*15*), which has arisen to supply the enormous amount of fat stored in the broad ligament. The dissections were performed at a time of year when the testes were retracted in the males, occupying a position in the body cavity corresponding to the ovaries in the female. The ligaments were distended with fat in the same manner and the blood vessels were found to occupy similar courses.

Just posterior to the ovarian arteries, the posterior mesenteric artery (*64*) arises from the aorta. A short distance farther back the iliolumbar arteries (*29*) are given off. Each of these, after giving off one or two slender branches (*32*) to the dorsal muscles, divides into two main branches (*30* and *31*). *30,* which we propose to term the 'lateral abdominal' branch, runs within the abdominal wall, anastomosing anteriorly with branches of the superior epigastric (*An.5*). It is confined to the dorsal and lateral portion of the abdominal wall. The other branch, *31*, which we propose to term the 'lateral cutaneous,' penetrates the abdominal wall not far from the origin of the iliolumbar from the aorta, and supplies the skin and panniculus adiposus of the side. It anastomoses with the superficial epigastric and the external mammary (*An.6*).

A fifth pair of lumbar vessels come off just behind the iliolumbars, and the aorta then bifurcates into the two common iliac arteries (*38*). Given off dorsally at the point of bifurcation is the medial sacral or caudal artery (*37*). This is hidden by the postcaval vein, which must be removed to see it.

The common iliac runs in a straight line laterally and posteriorly toward the thigh. After giving off the small internal iliac (*50*), which is deeply buried in the sacral muscles and extends along the side of the tail, the main stem will be known as the external iliac until it passes over the inguinal ligament to become the femoral (*56*). The external iliac gives off, just laterally to the internal iliac, a branch (*35*), the uterine, which runs in the broad mesentery of the ovary, supplying part of the fat-body and the wall of the oviduct which it follows closely in its course (*33*), and anastomosing with the 'ovarian' at *An.7*. The uterine gives off a posterior branch (*36*) to the median portion of the uterus, and a branch (*65*) to the lateral wall of the bladder. A small branch (*34*) came off dorsally to the musculature.

The external iliac now gives off an extensive branch (*43*), the deep femoral artery; this supplies, by a branch (*44*), the rectum (*49*), the anal glands (*51*), the vagina (*47–48*), and the midline of the bladder (*61*). The deep femoral continues as the common stem of the united proximal portions of the inferior epigastric (*52–60*) and the superficial epigastric (*53*).

The inferior epigastric courses ventral to the inguinal ligament and then forward, close to the midline, in the rectus abdominis muscle (*66, 66*). It anastomoses anteriorly with the superior epigastric, a continuation of the internal mammary, at *An.3*. The superficial epigastric (*53, 53*) runs ventrally in the subcutaneous tissue, not far from the midline, anastomosing with the 'lateral cutaneous' (*An.6*), and with the external mammary (*An.1*).

Below the junction of the superficial and the inferior epigastrics, a branch (*46*) is given off to the ventral muscles of the thigh.

Turning again to the external iliac, we find a vessel (*54*) which parallels the inferior epigastric in its course at a higher level in the body wall. This vessel runs along the inner side of the abdominal wall within the fascia. We propose to term it the 'abdominal circumflex'. It anastomoses anteriorly with the 'lateral abdominal' and the superior epigastric (*An.4*).

The multiplication of vessels supplying the body wall and the skin is worthy of note. The skin is supplied by two vessels occupying parallel courses—the superficial epigastric and the 'lateral cutaneous,' the former lying near the midventral line, the latter about halfway up the side of the animal. The abdominal wall itself is supplied by three parallel vessels, the 'lateral abdominal,' the 'abdominal circumflex,' and the inferior epigastric.

THE UNPAIRED VISCERAL BRANCHES OF THE ABDOMINAL AORTA (fig. 4)

The coeliac artery leaves the aorta close to the diaphragm (*3*). It divides into a number of branches a short distance from its origin from the aorta. Of these a branch (*5*) proceeds to supply the oesophagus (*22*) and the region of the lesser curvature of the stomach. This branch (*5*) is the left gastric or coronary artery. A splenic branch (*4*) then comes off from the coeliac and divides into two, one branch supplying the right end of the spleen and the other the left end, from which the latter sends off a number of vessels running mainly straight and parallel within the omentum to the greater curvature of the stomach (*6*). Arriving at the greater curvature, these vessels, the left gastroepiploics (*7*), form by anastomosis a longitudinal trunk which runs along the line of attachment of the omentum to the greater curvature and supplies the adjacent stomach wall.

The hepatic artery (*2*) is unusually long and of small diameter. It arises from the main stem of the anterior pancreaticoduodenal (*8*) and proceeds forward to the liver within the hepatoduodenal ligament in company with the portal vein and the common bile duct. It gives off a small cystic artery (*28*), which pursues a tortuous course to the gall bladder, over whose surface it spreads out into a network of arterioles, very conspicuous when injected with yellow.

The anterior pancreaticoduodenal artery (*8*) describes a wide sweep along the inner side of the duodenum, running in the pancreatic tissue to which it gives off branches. It anastomoses posteriorly with the posterior pancreaticoduodenal (*9*), from the superior mesenteric trunk. The superior mesenteric (*10*) comes off from the aorta in some cases close behind the coeliac (fig. 3, *10*), in other cases farther back, as in figure 4.

The superior mesenteric gives off, first, a long vessel (*12, 12*) which supplies the 'free compound limb'[4] composed of the descending portion of the colon (*36*) and the commencing portion of the rectum (*37*). The homologies of this vessel are difficult to determine. It may correspond to the descending branch of the middle colic in man, which has become shifted from the rest of the middle colic to acquire a separate opening into the superior mesenteric. This is suggested by the fact that it communicates by anastomosis (*An.C.*) with the posterior right colic, but since the portion of the intestine referred to as the 'free compound limb' appears to be an unusual development, the nomenclature of the vessel (*12*) is not otherwise attempted.

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[4] The colon is in the form of an inverted V, as seen in figure 4 and figure 10, and thus consists of an obliquely ascending limb and an obliquely descending limb, it being difficult to identify a transverse limb. The rectum is long, in the form of a wide inverted U, the ascending limb of which is closely applied to the descending limb of the colon. These two closely applied portions of the gut form a characteristic limb of the intestine, the mesenterial attachment of which is anterior to the point where the two halves of the limb separate. Thus the limb is free in the sense that it can move considerably in the abdominal cavity.

Further on the superior mesenteric gives off two vessels supplying the sacculated portion of the colon, and it seems safe to term them the posterior (*14*) and anterior (*15*) right colics.

The posterior pancreaticoduodenal artery (*9*) runs forward from the superior mesenteric to anastomose with the anterior pancreaticoduodenal artery. The superior mesenteric then gives off the middle colic artery (*21*) which runs at first forward along the ascending portion of the rectum (*37*). This vessel then turns round along the transverse and descending portions of the rectum (*38*) to anastomose with the left colic (*20*) from the inferior mesenteric. The intestinal arteries (*11*) arise in a group and spread out to supply the free portion of the small intestine. They communicate by anastomoses near the line of attachment of the mesentery before ramifying in the wall of the gut. The main stem of the superior mesenteric continues on as the ileocaecocolic artery (*13*). This runs to the ileocolic junction and supplies the caecum and adjacent parts of the ileum and colon.

THE ANTERIOR VEINS (fig. 5)

Right and left precavals are separately developed and enter the right auricle independently, as is usual among rodents. The right precaval receives the azygos vein (fig. 6). Each precaval receives the internal mammary of its side (*36*), which runs forward along the internal wall of the chest slightly lateral to the midventral line (fig. 6) and also the supreme intercostal (*35*).

The arrangement of the veins in the head and neck is variable. Figure 5 is a catalogue of the veins and their distribution as they appeared in one particular dissection. In the description that follows attention will be drawn, however, to the constant features.

In the first place, the main trunk of the precaval divides into two branches of approximately equal diameter just posterior to the clavicle. One of these passes dorsal to the clavicle, the other ventral and superficially, and by subsequent anastomosis of the two branches in front of the clavicle a venous circle is formed around the pectoral girdle on each side. This venous circle is invariably present and is very characteristic. The double channel afforded to the flow of blood returning from the head is undoubtedly correlated with the needs of the circulation in the animal, the distortion of the neck and chest region through the animal's habit of curling up in the median plane during rest and hibernation[5] probably setting up pressures in the neighborhood of the clavicle that would otherwise impede the flow of blood. It is evident that the original course of the precaval lay along the dorsal half of the venous circle and that the ventral half is a new development. This is shown by the fact that in other vertebrates the normal position of the precaval is within the pectoral girdle and not superficial, and also by the occurrence of several large veins (*26, 25, 27, 22*) opening into the dorsal limb, while the ventral limb is comparatively devoid of branches. Particularly does the occurrence of the vertebral vein (*22*) reveal that the dorsal limb represents the original trunk of the precaval.

Into the anterior portion of the venous circle two large trunks open close together, coming from the side of the head, one ventral to the other (*21* and *20*). Their distribution shows them to represent the anterior and posterior facial veins, respectively. The fact that the two facial veins open always independently into the venous circle suggests that at least the front end of the venous circle is equivalent morphologically to the external jugular vein. Indeed, it would be easy to suggest that the whole of the ventral limb of the ring is simply the external jugular, up to the posterior facial, and that the dorsal limb represents the internal jugular, the two anastomosing to form a venous circle some distance in front of their original separation. This is the more plausible when it is remembered that in man a connection exists between the external jugular and the common facial, which arises from the internal jugular. On this view the position of the vertebral would not be a serious difficulty, since its point of origin might have been shifted with regard to the main trunks. The position of the clavicle, however, remains a difficulty, since, in man, it does not run in the fork between the external and internal jugular, both passing dorsal to it.

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[5] During sleep or hibernation the animal assumes an attitude of extreme flexion, the head being bent between the forelegs.

The vessel (*14*) was decided to be the internal jugular from its distribution. The main trunk of *20* would have to be regarded as internal jugular, and this would accord with the view suggested above. The fusion between external and internal jugular would then, however, have to extend up the posterior facial to the origin of *14*. On any other view the position of the internal jugular among the branches of the posterior facial would be an anomaly.

A transverse connection between the jugulars of right and left sides was not found in any specimen dissected.

THE AZYGOS VEIN (fig. 6)

The azygos vein arises posteriorly at the level of the diaphragm by the fusion of the small right and left ascending lumbar veins (*7* and *8*). It then pursues a straight course forward within the mediastinum, closely apposed to the vertebral column to the right of the midline; that is to say, its course is a continuation of the right ascending lumbar. There is no hemiazygos, the left intercostals running across the ventral surface of the spinal column to open into the azygos at points opposite the entrance of the right intercostals. The intercostals course along the posterior borders of the ribs, emerging from the intercostal muscle close to the tuberculum of the rib. The anterior intercostals make a distinct bend backward, after leaving the border of the rib, to join the azygos. On both sides the last nine intercostals open into the azygos, the first two of the nine on the left entering by a common stem (*9*). The first three intercostals open into a supreme intercostal on each side (*3* and *11*). The supreme intercostals run forward to the precavals.

About halfway down the side of the chest from the middorsal line the intercostals anastomose with the rib branches of the internal mammaries.

THE POSTCAVAL VEIN (fig. 7)

At its posterior end the postcaval runs dorsal to the aorta, but farther forward it comes to lie ventral to the aorta and is markedly displaced by the presence of the left kidney to the right of the midline. This displacement can be seen in figure 7 opposite the left kidney. Anteriorly, the postcaval runs again in the midline, pursuing a straight or only slightly tortuous course to the right auricle.

The vessels (*10*) run from the fat-distended mesentery of the gonad. Figure 7 is taken from a male specimen in which the testes are retracted and occupy positions with regard to the blood vessels and the fat mesentery similar to the female. The veins *10* then will be referred to as the 'spermatics,' but it is to be noted that a slender offshoot (*22*) ran directly from the left iliolumbar vein to the left testis. This instance is parallel to the case of the offshoot *27* (fig. 3, p. 172) which was discussed in the case of the female arterial system. The presence of a vessel, however, much reduced running to the gonad from some portion of the central vascular system anterior to the vessels that supply the fat-body suggests that the latter vessels have usurped the place of the original ovarians or spermatics.

The postcaval receives a series of segmentally arranged lumbar veins (*9*)[6] entering in five pairs upon the dorsal side of the postcaval. The left renal (*27*) is fairly long and curves forward before entering the postcaval. Near the hilus of the left kidney a pair of small veins (*18* and *20*) enter the left renal from the anterior and posterior regions of the fat-mass packed along the medial edge of the kidney. The anterior of these tributaries (*18*) also drains the left adrenal gland. Passing to the right kidney, it is found that the same relationship of blood vessels holds save that the right adrenal is drained by an independent vein (*14*) which opens directly into the postcaval.

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[6] These lumbar veins are usually paired, but in some cases they are represented by median tributaries entering the postcaval in the middorsal line, and bifurcating some distance from it.

There are now to be considered, on each side of the body, the three conspicuous vessels (*6, 7, 8*) on the right and (*15, 17, 19*) on the left, which drain the dorsal region of the abdominal wall and receive tributaries from the epaxial muscles of the back and, in their distal portions, from the skin. These vessels tend to associate themselves with the renal veins. Their actual disposition varies from specimen to specimen, but it can be seen in figure 7 how the first one on the right (*6*) opens into the right renal and the first two on the left (*15* and *17*) open by a common stem (*25*) into the left renal, and in this instance this common stem is drawn back some distance past the origin of the most posterior of the series (*19*) from the postcaval. The term 'lateral lumbar' is suggested for these veins. As has been mentioned, they closely accompany the corresponding 'lateral lumbar' arteries (fig. 3, p. 171).

The iliolumbar veins (*21*) are described in the next section. The hepatic veins are illustrated on the left of the postcaval, the liver having been pulled over to the left to expose them. The postcaval runs through the caudate and right posterior lobes of the liver, close to their dorsal edge, to reach the diaphragm, which it penetrates in the left portion of the central tendon, receiving from the diaphragm the phrenic veins (*1*).

THE POSTERIOR VEINS (fig. 8)

The postcaval takes origin posteriorly from the fusion of the right and left common iliac veins (*30*) together with the caudal vein (*13*), which runs axially, entering the postcaval in the midline. About an inch and a half in front of this point the postcaval receives on each side a vein (*3*) from the fat-distended mesentery of the gonad. *3* is termed the 'ovarian,' as in the case of the arteries. One of its branches (*31*) comes from the ovary (*25*). The 'ovarian' anastomoses with the branch (*18*) from the iliac—the uterine. *12,* a branch of the uterine, comes from the bladder and corresponds to *65*—the lateral vesicular artery—in figure 3. Into the uterine enter veins from the oviduct and the median portion of the uterus (*32*).

In front of the 'ovarians' the iliolumbar veins enter (*2*), which bring blood from the abdominal wall and the skin. Tracing one of these vessels outward from the postcaval it will be seen from the figure that it passes ventrally over the thick muscle at the side of the vertebral column and then is seen by transparency through the fat-mesentery (*26*), as is indicated by the dotted lines. At about the line of attachment of the mesentery to the dorsal body wall it divides into two branches of equal size (*5* and *9*) and gives off a minor branch (*10*) to the dorsal muscles. The branches (*5* and *9*) pursue nearly parallel courses, *5* running in the body wall and *9* in the skin. (It will be noted that a more extensive portion, proximally, of *9* is dotted to indicate that it is hidden from view, in ventral aspect, by the abdominal wall, which it penetrates at about the line of attachment of the fat-mesentery of the ovary.) In accordance with the nomenclature previously adopted in the case of the arteries, we propose to refer to *9* as the 'lateral cutaneous,' and to *5* as the 'lateral abdominal.' The 'lateral abdominal' drains the body wall dorsally and laterally and anastomoses with the inferior epigastric (*24*) posteriorly, with the superior epigastric (*6*) anteriorly, and also with the 'abdominal circumflex' (*29*) posteriorly and laterally. The 'lateral cutaneous' (*9*) anastomoses with the superficial epigastric (*11*) and with the external mammary (at *8*).

The branches of the common iliac vein

The internal iliac (*15*) enters the common iliac after running forward from the side of the tail parallel to and not far from the caudal. The large blood supply of the tail may be associated with its large size and the considerable development of hair and of arrector pili muscles. The median position of the caudal vein is invariable. The vessel *28* is to be regarded as the deep femoral and receives a number of branches, as shown near its base. These branches are variable and they may enter the iliac directly by one or two independent roots. The haemorrhoidal vein (*27*) comes from the anus and rectum. In the cat and the rabbit this vessel enters the internal iliac. The haemorrhoidal vein is associated with branches *14* from the deep muscles of the thigh, the common stem constituting the main axis of the deep femoral. The deep femoral receives the inferior and superficial epigastrics (*24* and *11*, respectively). The uterine (*18*) and the vesicular (*12*) enter jointly at the base of the deep femoral.

The 'abdominal circumflex' enters the external iliac from the posterior lateral portion of the abdominal wall (compare p. 174).

THE HEPATIC PORTAL SYSTEM (fig. 9)

The branches of the hepatic portal vein are as follows:

1. The anterior pancreaticoduodenal vein (*20*), which drains the greater part of the right half of the duodenal loop together with the adjacent pancreatic tissue. It anastomoses behind with the posterior pancreaticoduodenal (*18*).

2. The right gastroepiploic (*1*), which drains the pyloric canal wall and a considerable portion of the fundus of the stomach, eventually reaching the greater curvature and joining with the left gastroepipolic (*6*).

3. The gastrosplenic (*2*) which runs in the splenic lobe of the pancreas from which it receives small vessels. The gastrosplenic receives blood from the spleen by two vessels, the left (*8*) and right (*9*) splenics. These two vessels are connected in the spleen by a large cross-channel into which, at the points of communication with the splenic veins, open, *a*) the left gastroepiploic (*6*) from the greater curvature of the stomach—the left gastroepiploic runs in the great omentum

which stretches across to become continuous with the fibrous capsule of the spleen; and, *b*) the middle gastroepiploic (*7*) from the dorsal side of the stomach.

4. The left gastric or coronary vein (*3*) enters the portal vein near the base of the gastrosplenic or it may enter the latter directly.

5. The inferior mesenteric vein (*19*) comes from the descending portion of the rectum.

6. A vessel which is designated as *X* and which supplies the 'free compound limb' of the intestine. This corresponds with the vessel *12,* figure 3, which was discussed in the arteries (p. 175). In the case of the veins, however, this vessel is seen to drain a portion of the gut (namely, the descending colon and the commencing part of the rectum), which in the rabbit and cat is drained solely by the inferior mesenteric. Therefore, it would appear that the vessel *X* is equivalent to a branch of the inferior mesenteric which has become separated from the parent stem. A development of this nature is presumably to be correlated with the occurrence of the 'free limb' of the intestine, which, as explained previously, is a special feature in the anatomy of the animal (footnote 4, to page 175).

7. The main stem of the portal vein is now to be regarded as the superior mesenteric trunk proper. It receives blood from the remaining portions of the intestine.

8. The posterior pancreaticoduodenal vein (*18*) collects blood from the remainder of the duodenal loop and pancreas.

9. Numerous intestinal veins (*14*) converge in the mesentery from all portions of the free intestine. Their region of convergence is surrounded by masses of lymphoid tissue, the pancreas Aselli.

10. The caecum is drained by the ileocolic vein (*11*) which receives anterior (*10*) and posterior (*12*) branches.

SUMMARY

Right and left precaval veins are independently developed. The common carotids are associated with the right subclavian artery into a common trunk from the aortic arch. Upon the precaval of either side there is developed a venous circle by anastomosis around the clavicle, affording a double passage to the flow of blood. Significance and derivation discussed. From abdominal aorta and postcaval three pairs of vessels run out laterally and posteriorly into the dorsal abdominal wall. They are associated at their bases into common trunks, the manner of the association being variable and asymmetrical, but at least one of the three comes off from the renal vessel. Correlated with the development of the broad ligament as a reservoir of fat, a pair of vessels arise from aorta and postcaval, supplying the fat and the gonads, and anastomosing with the uterine vessel or the vessel of the vas deferens. Certain anomalies in their relationships raise the question as to whether they are to be regarded as the true spermatics. The iliolumbars are extensive vessels. In the abdominal region there is a multiplication of vessels that parallel the epigastrics at higher levels in the body wall and skin. Hemiazygos not developed. The development of a free mesenterial limb of large intestine is associated with a special branch of the superior mesenteric artery and a branch of the portal vein that cannot be assigned to normal association either with superior or inferior mesenterics, but appears to represent a branch of inferior mesenteric detached from parent stem.

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PLATES

PLATE 1

EXPLANATION OF FIGURE

The vessels arising from the aortic arch and the branches of the right common carotid artery

1, aortic arch
2, brachiocephalic or innominate
3, left subclavian
4, common stem of the right common carotid and the right subclavian
5, left common carotid
6, right common carotid
7, right subclavian
8, right internal mammary
9, internal carotid
10, occipital
11, thyroid
12, laryngeal
13, 14, 15, the three branches into which the external carotid divides
16, external maxillary
17, external carotid
18, to the parotid gland
19, to a cervical lymph node
20, internal maxillary artery
21, to the pharynx
22, to the root of the tongue
23, to the submaxillary gland
24, to the masseter
25, to the floor of the mouth
26, lingual artery and its branches
27, to the sublingual gland
28, left internal mammary
29, to the angle of the jaw
31, right ventricle
32, right auricle
33, pulmonary artery; *35, 36,* its right and left branches
34, left auricle
37, ligamentum arteriosum

PLATE 1

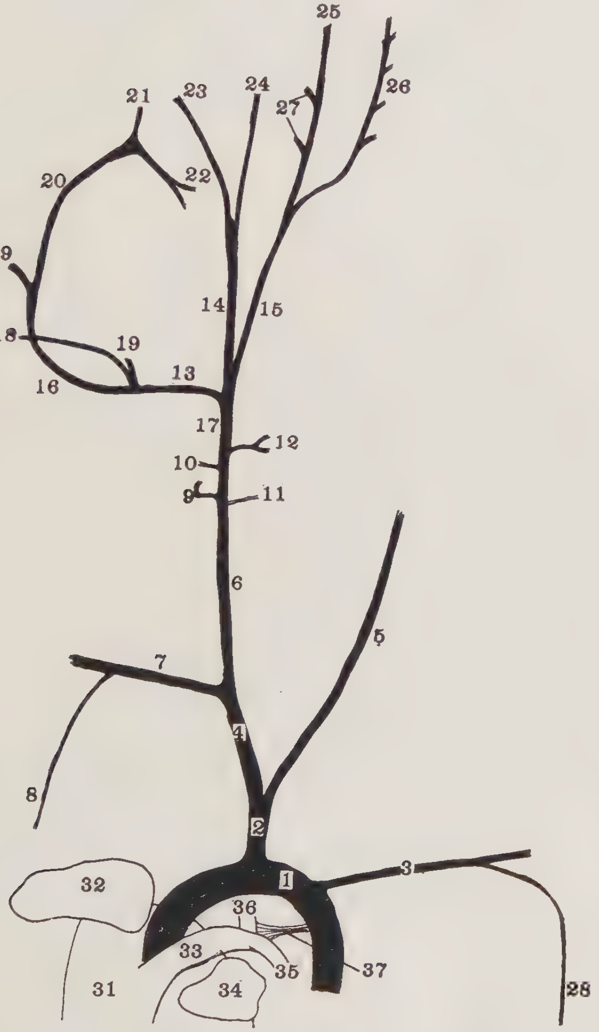


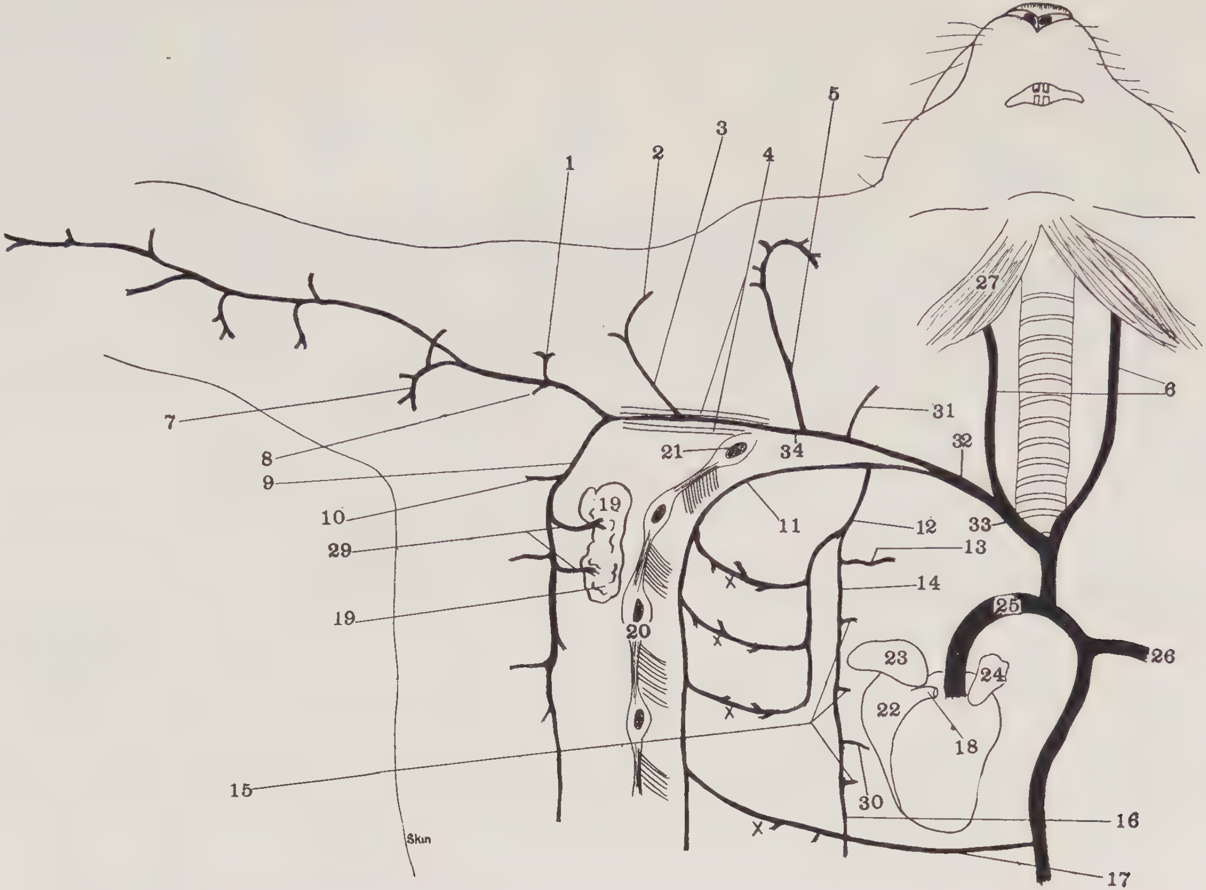
PLATE 2.

EXPLANATION OF FIGURE

The branches of the right subclavian artery

1, a small branch to an axillary lymph node
2, to the head of the humerus
3, branch of axillary (subscapular?)
4, nerves of the brachial plexus
5, branch of the axillary running to the region of the clavicular venous circle (see branches of the precaval) which it parallels for a portion of the course of the venous circle
6, right and left common carotids
7, to flexor muscles of arm
8, to the latissimus dorsi muscle (thoracodorsal artery?)
9, external mammary
10, to pectoralis major (ventral thoracic artery?)
11, internal mammary
12, supreme intercostal
13, to thymus
14, common stem of oesophageal and bronchial arteries
15, bronchials
16, oesophageal
17, first intercostal (arising directly from the thoracic aorta)
18, common pulmonary artery
19, mammary gland
20, wall of chest
21, first rib
22, right ventricle
23, right auricle
24, left auricle
25, aortic arch
26, left subclavian
27, sternohyoid muscle
29, branches of external mammary supplying mammary gland
30, mediastinal artery
31, vertebral
32, left subclavian
X,X,X,X, anastomoses of the branches of the internal mammary with the branches of the supreme intercostal and first intercostal. The heart has been pulled over to the right to show the arch of the aorta

PLATE 2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X
Skin

PLATE 3

EXPLANATION OF FIGURE

The abdominal aorta and the arteries of the posterior region of a female in ventral aspect

The dissection illustrates the double blood supply, by anastomosis of arteries arising anteriorly and posteriorly, of the skin, of the body wall, and of the fat-bearing ligament of the gonad. The multiplication of vessels that parallel the epigastrics is indicated. The rectum and the vagina, and the arteries supplying them, have been exposed by cutting through the pubic symphysis and bending apart the halves of the pelvic girdle. A portion of the rectus abdominis muscle has been cut away, leaving the inferior epigastric artery which runs in the rectus, stretched alone across the femoral artery which is further exposed by a slit in the skin of the thigh. The skin is necessarily intact in one place over the thigh to show the superficial epigastric artery.

1, external mammary
2, internal mammary
3, intercostals
4, bifurcation of right renal at hilum
5, *5*, adrenal glands
6, adrenal artery
7, left phrenic
8, coeliac
9, right phrenic
10, anterior mesenteric
11, lumbar arteries
12, 'first lateral lumbar' of left
13, common stem of 'lateral lumbars' of right
15, *15*, 'ovarians'
16, *16*, renal arteries
17, *17*, branch of renal to fat in posterior region of kidney
18, branch of first right 'lateral lumbar' to epaxial muscles of back
19, *20*, first right 'lateral lumbar'
21, common stem of *22*, second, and *23*, third, right 'lateral lumbars'
24, common stem of *25*, second, and *26*, third, left 'lateral lumbars'
27, offshoot of left renal which anastomoses with left ovarian (see text)
28, anterior branch of 'ovarian'
29, iliolumbar
30, 'lateral abdominal'
31, 'lateral cutaneous'
32, minor branches to expaxial muscles
33, ovarian branch of uterine
34, minor dorsal branch
35, uterine
36, branch of latter to median portion of uterus
37, sacral
38, left common iliac
39, uterus
40, bladder
41, *42*, vagina and glands
43, deep femoral
44, branch to rectum and vagina
45, stem of inferior and superficial epigastrics
46, to thigh muscle
47, *48*, to vagina
49, to rectum
50, right internal iliac
51, anal glands
52, flexion of inferior epigastric
53, superficial epigastric
54, 'abdominal circumflex'
56, femoral
57, *58*, *59*, branches of femoral to leg
60, inferior epigastric
61, to midline of bladder
62, ovary
63, horn of uterus
64, posterior mesenteric
65, lateral vesicular
66, rectus abdominis
An., anastomosis
An.1, external mammary and superficial epigastric
An.2, external mammary and 'lateral cutaneous'
An.3, internal mammary and inferior epigastric
An.4, internal mammary and 'abdominal circumflex'
An.5, internal mammary and 'lateral abdominal'
An.6, 'lateral cutaneous' and superficial epigastric
An.7, uterine and 'ovarian'

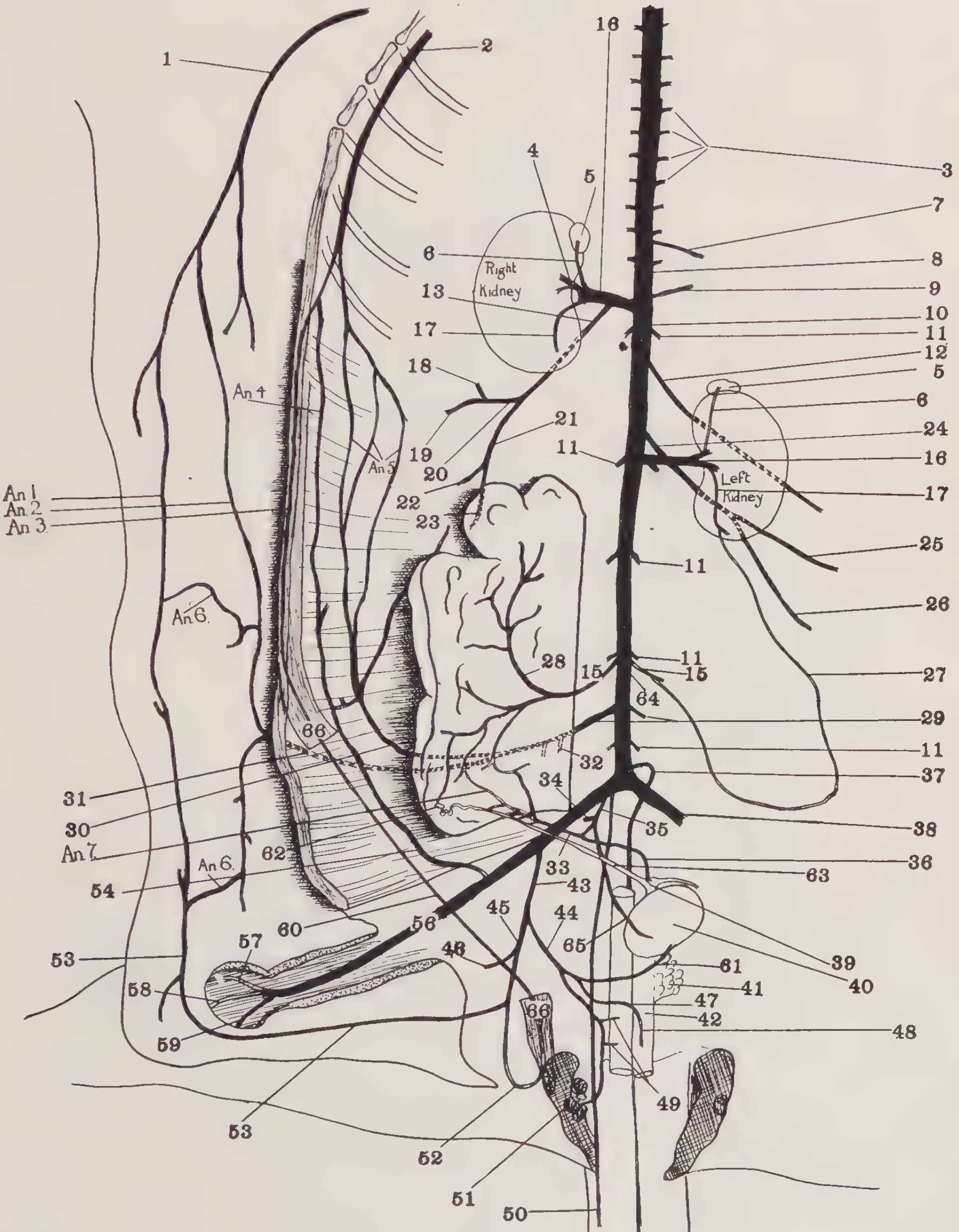
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PLATE 4

EXPLANATION OF FIGURE

The unpaired visceral branches of the abdominal aorta
Right ventral aspect. The gut has been placed on the stretch

1, the right renal artery and its somatic branches
2, hepatic artery
3, coeliac
4, splenic
5, left gastric (coronary)
6, greater curvature of stomach
7, left gastroepiploics
8, anterior pancreaticoduodenal
9, posterior pancreaticoduodenal
10, superior mesenteric
11, intestinals
12, branch to the compound free limb of the intestine (see text)
13, ileocaecocolic
14, posterior right colic
15, anterior right colic
16, caecal
17, inferior mesenteric
18, superior haemorrhoidal
20, left colic
21, middle colic
22, oesophageal artery and oesophagus
23, cystic lobe of liver
24, left median lobe of liver
25, left lateral lobe of liver
26, stomach
27, pylorus
28, cystic artery and gall bladder
29, right kidney
30, right lateral lobe of liver
31, spleen
32, duodenum
33, mesenterial portion of small intestine
34, caecum
35, ascending colon
36, descending colon
37, ascending portion of rectum
38, descending portion of rectum
39, anus
An.c., anastomosis of (*12*) with posterior right colic

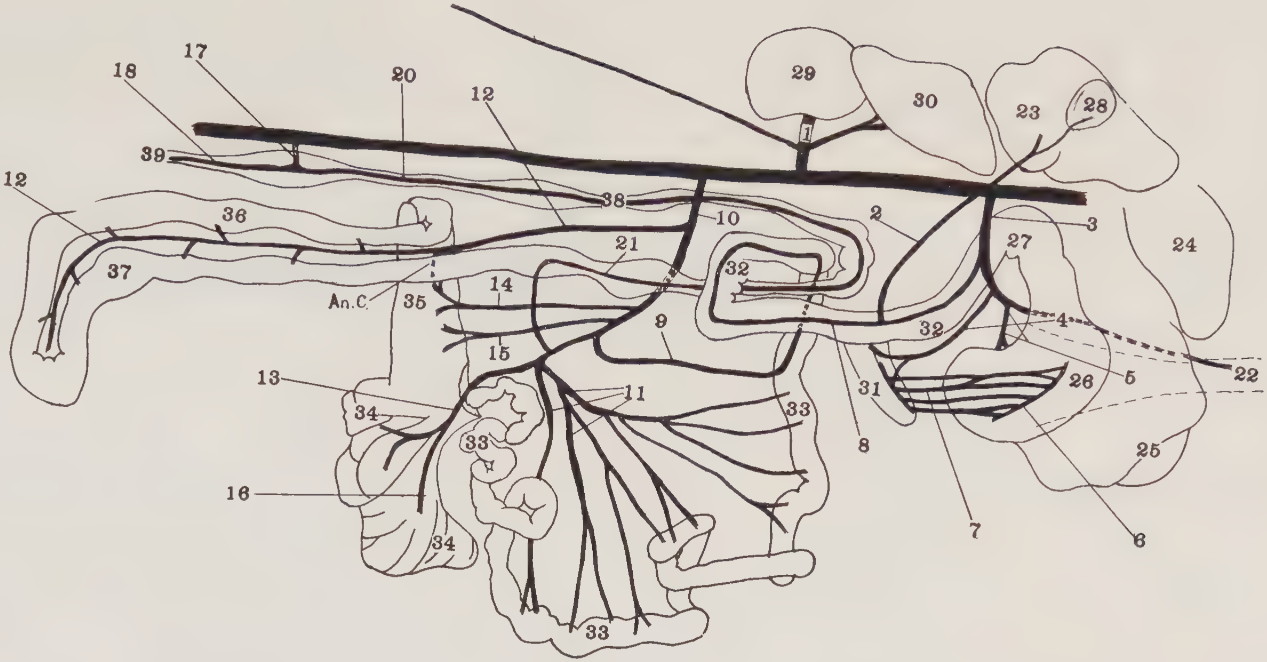
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PLATE 5

EXPLANATION OF FIGURE

The left precaval vein and its branches

View of a dissection of the anterior veins in left ventral aspect. The internal mammary (*36*) is shown in its natural position relative to the other vessels. The long thoracic (*33*) and its continuation, the external mammary (*34*), are shown below, the skin containing them having been pinned out. The nomenclature of the peripheral branches is not attempted; they are indicated below according to the parts that they supply.

1, from the lower lip
2, the cheek sac
3, the mandible
4, the floor of the orbit (inferior ophthalmic vein)
5, the upper eyelid
6, scalp
7, tongue (lingual vein)
8, masseter
9, masseter
10, submaxillary gland
11, region of the skin ventral to the pinna
12, maxilla and teeth sockets of upper jaw
13, parotid gland
14, internal jugular vein
15, internal ear
16, parotid gland
17, sublingual gland
18, a cervical lymph node
19, a cervical lymph node
20, posterior facial vein
21, anterior facial vein
22, vertebral vein
23, muscles of pharynx
24, thyroid vein
25, from the epaxial muscles of the neck
26, muscles of shoulder
27, from the anterior border of the upper arm
28, from the subcutaneous tissue of the axillary fossa
29, cephalic vein
30, brachial vein
31, subclavian vein
33, long thoracic vein
34, external mammary vein
35, left supreme intercostal vein
36, left internal mammary vein
37, superior epigastric vein
38, left precaval vein
39, coronary veins

Between the index figures *30* and *31* the chest wall and an axillary lymphatic vessel are indicated.

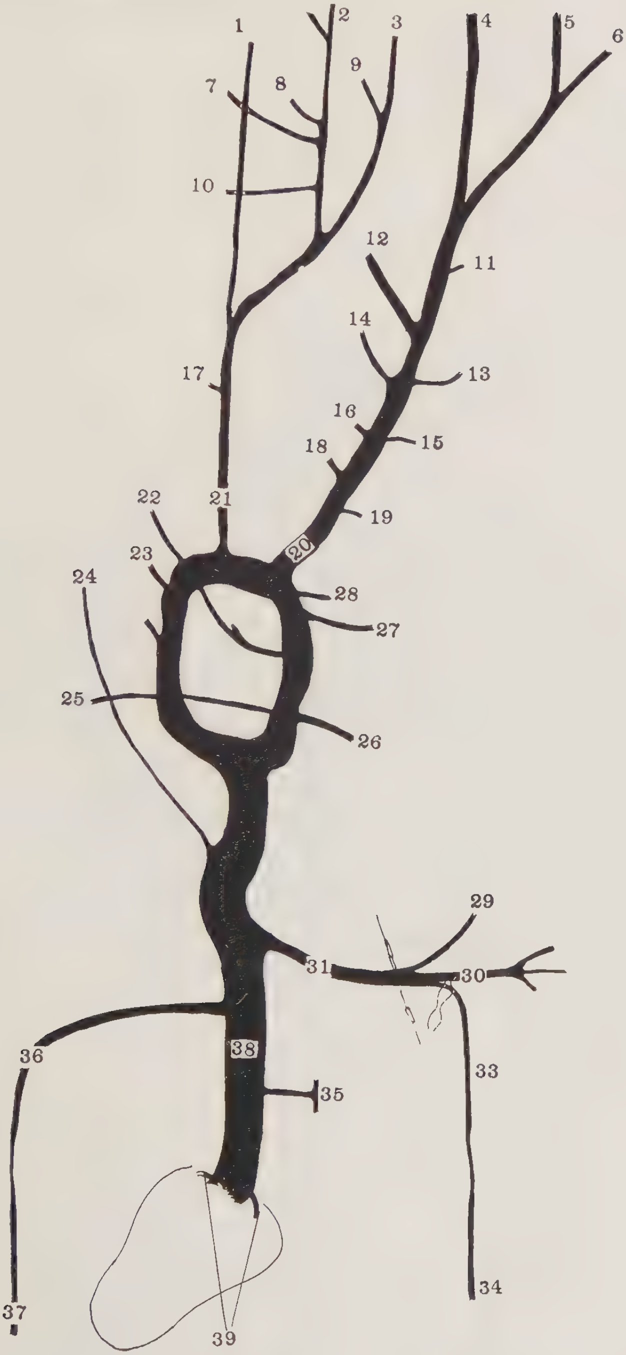
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PLATE 6

EXPLANATION OF FIGURE

The azygos vein

Internal view of the dorsal wall of the thorax. The chest wall has been cut through by a longitudinal incision about half an inch to the left of the midventral line, and the larger right-hand flap thus formed has been pulled to the right and bent so as to reverse the concavity of the ventral portion of the chest wall, thus exposing the right and left internal mammaries running close together. The branches of the right internal mammary in the figure are observed to bifurcate at the junction of the vertebral and sternal portions of the ribs and then anastomose with the intercostals.

The heart has been turned over in a forward direction and the postcaval pulled out of view to the left.

1, right precaval
2, right internal mammary
3, right supreme intercostal
4, left internal mammary
5, midventral line of sternum
6, ensiform plate of sternum
7, right ascending lumbar
8, left ascending lumbar
9, common stem of fourth and fifth intercostals of left side
10, azygos vein
11, left supreme intercostal
12, left precaval
13, postcaval

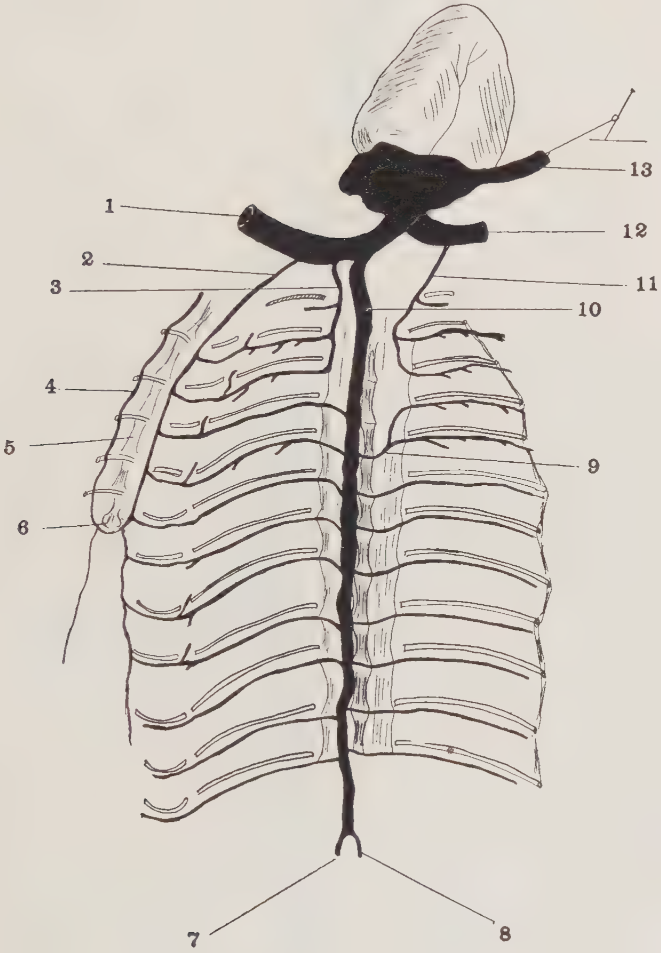
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PLATE 7

EXPLANATION OF FIGURE

The postcaval vein. Ventral aspect. Male

1, phrenic veins
2, adrenal gland (right)
3, branch of renal vein to fat apposed to kidney (on right)
4, right renal vein
5, branch of right renal vein to fat apposed posteriorly to kidney
6, 'first lateral lumbar' of right
7, 'second lateral lumbar' of right
8, 'third lateral lumbar' of right
9, paired lumbar veins
10, spermatics
11, right and left common iliacs
12, central tendon of diaphragm
13, hepatic veins
14, right adrenal vein
15, 'first lateral lumbar' of left
16, left adrenal gland and vein
17, 'second lateral lumbar' of left
18, branch of renal to fat and to adrenal gland
19, 'third lateral lumbar' of left
20, branch of left renal to fat
21, iliolumbar
22, offshoot to testis (see text)
23, testis (retracted)
24, common stem of second and third 'lateral lumbars' of right
25, common stem of first and second 'lateral lumbars' of left
26, caudal vein
27, left renal

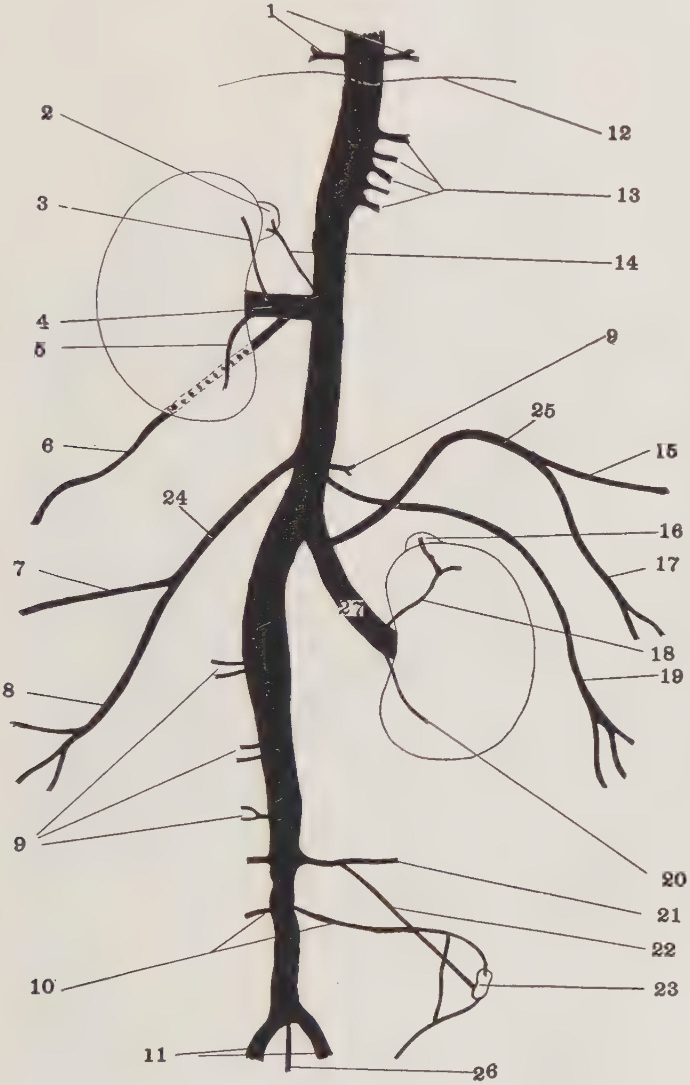
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PLATE 8

EXPLANATION OF FIGURE

The posterior veins in ventral view

The body wall has been separated from the skin and raised up, while the skin has been pinned out on the stretch, so that the veins that course in both these layers can be indicated. The fat-bearing ligament of the gonad is shown within the concavity of the body wall.

1, postcaval vein
2, iliolumbar vein
3, ovarian
4, from epaxial muscles of back
5, 'lateral badominal' vein
6, superior epigastric
7, anastomosis of superior and inferior epigastrics
8, anastomosis of 'lateral cutaneous' and superficial epigastric
9, 'lateral cutaneous'
10, from epaxial muscles of back
11, superficial epigastric
12, vesicular vein
13, caudal vein
14, from the deep muscles of the thigh
15, internal iliac
16, enters from within the fascia of a groin muscle (the vastus medialis)
17, from the gluteal region
18, uterine vein
19, from the deep muscles of the thigh
20, femoral vein
21, from deep muscles of shank
23, from along the tibia
24, inferior epigastric
25, ovary
26, edge of fat-bearing ligament of gonad
27, haemorrhoidal vein
28, deep femoral
29, 'abdominal circumflex'
30, common iliac veins
31, branch of ovarian supplying ovary direct
32, branch of uterine to median portion of uterus

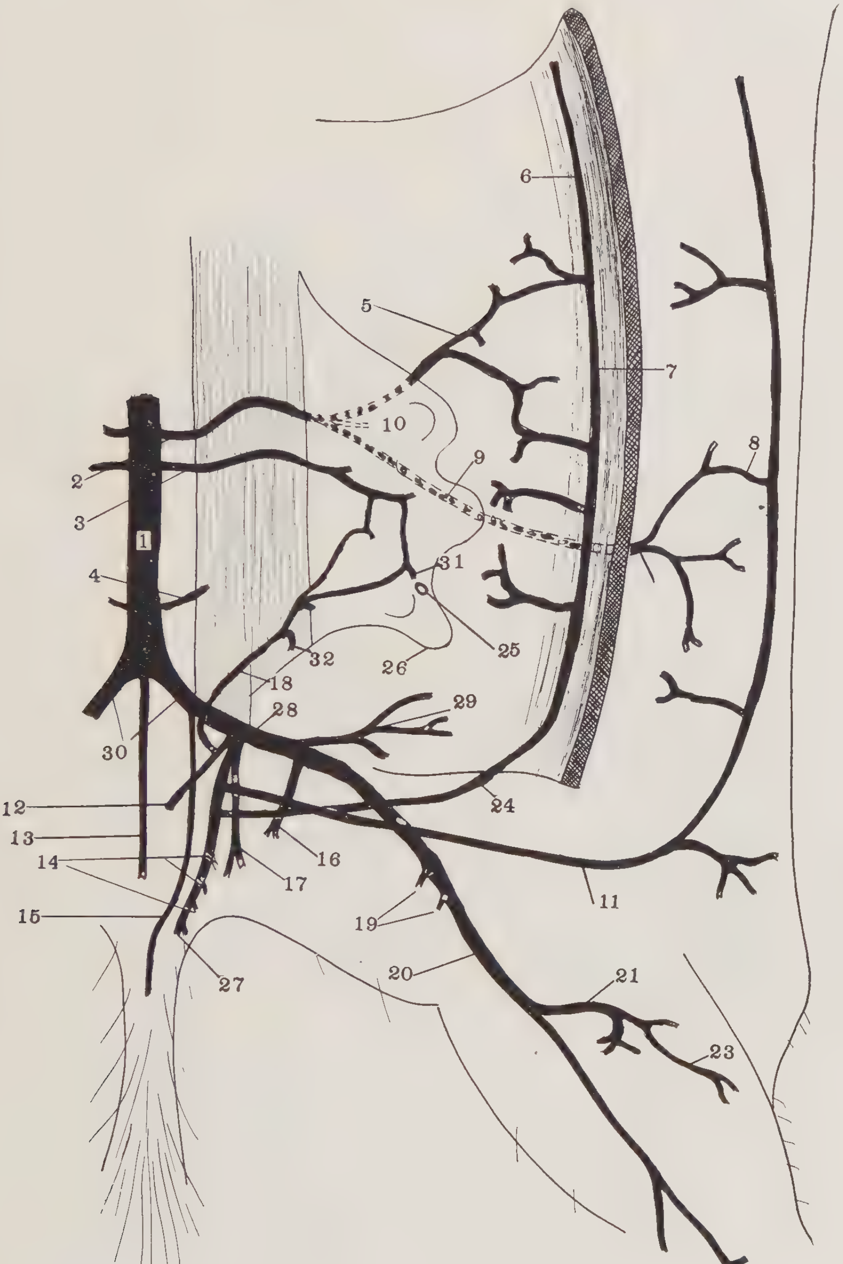
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PLATE 9

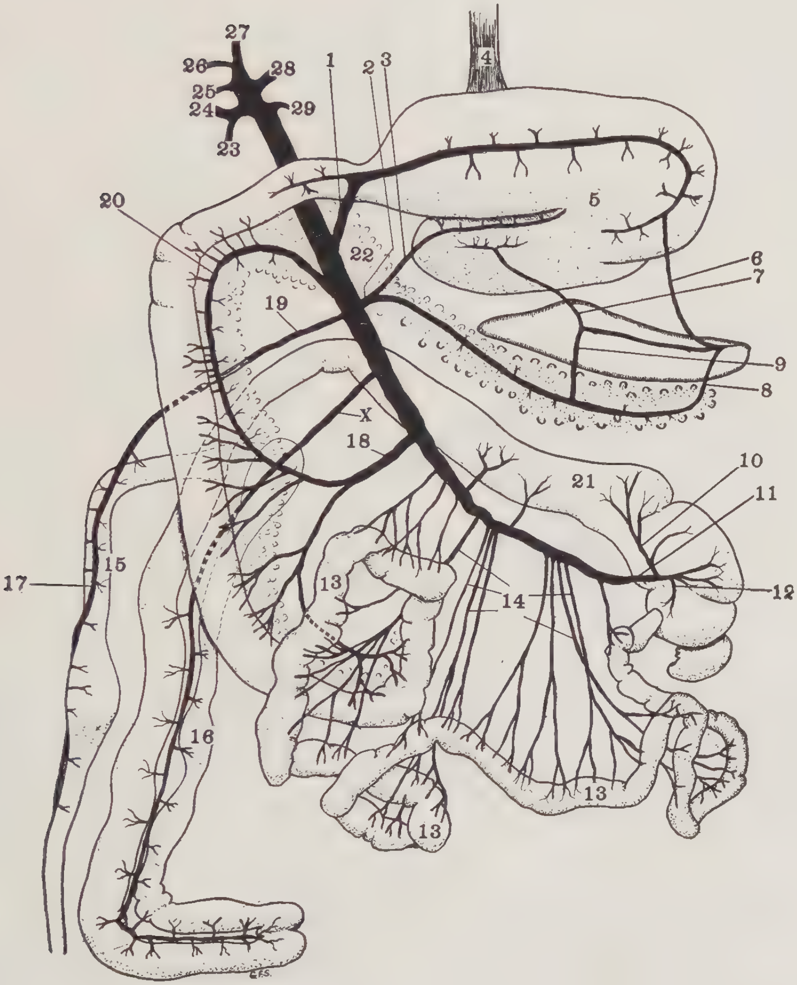
EXPLANATION OF FIGURE

The hepatic portal system. Ventral aspect

The intestine has been pulled over to the left and the stomach drawn forward by its fundus so that it is seen in keel view. The drawing was made from the right ventral aspect so that the descending portion of the rectum, held by its short mesentery against the dorsal body wall, is seen on the left of the figure.

The spleen is seen opposite figure *9* in the plate.

1, right gastroepiploic vein
2, gastrosplenic
3, coronary
4, oesophagus
5, stomach
6, left gastroepiploic
7, middle gastroepiploic
8, left splenic
9, right splenic
10, anterior ileocolic
11, ileocolic
12, posterior ileocolic
13, *13*, mesenterial portion of small intestine
14, intestinal veins
15, descending portion of rectum
16, rectum, ascending portion
17, inferior mesenteric vein, distal portion
18, posterior pancreaticoduodenal
19, proximal portion of inferior mesenteric
20, anterior pancreaticoduodenal
21, colon
22, pancreas (stippled)
23, afferent portal branch to caudate lobe of liver
24, afferent portal branch to right posterior lateral lobe
25 and *26*, afferent portal branch to right median lobe
27, afferent portal branch to left median lobe
28, afferent portal branch to left lateral lobe
29, afferent portal branch to Spigelian lobe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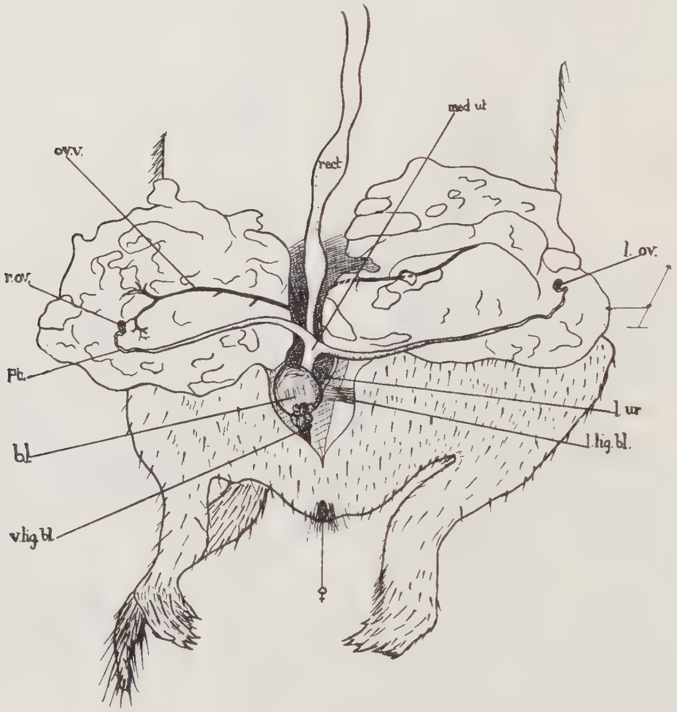

Dissection of a female, to show the broad ligaments of the ovaries distended with fat. It is to be noted that the ligament extends anteriorly to the ovary for a considerable distance and also in front of its line of attachment. The fat-distended mesenteries are the most conspicuous objects in the abdominal cavity, and they readily protrude when an incision is made in the abdominal wall.

ABBREVIATIONS

bl., bladder
F.t., Fallopian tube
l.lig.bl., left lateral ligament of the bladder
l.ov., left ovary
l.ur., left ureter
med.ut., median portion of uterus
ov.v., ovarian vein
rect., rectum
r.ov., right ovary
v.lig.bl., ventral ligament of the bladder (distended with fat)
♀, female aperture

THE OSSICLE AT THE STERNAL END OF THE CLAVICLE IN THE ALBINO RAT; THE HOMOLOGUE OF THE STERNAL EPIPHYSIS OF THE CLAVICLE IN MAN

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TWO FIGURES

The purpose of this note is twofold: 1) to make a record of two small bones, occurring in the skeleton of the albino rat, which until recently have not been mentioned by investigators studying the skeleton of this animal, and, 2) to suggest the possible homology of these bones with the so-called epiphyses of the clavicles in man.

These bones were observed in the albino rat by Donaldson and Conrow in their studies on the skeleton, but not included in their data (Donaldson, '24), and were also shown by Green ('24) in a drawing exhibited at the fortieth session of the American Association of Anatomists. They are probably the suprasternalia mentioned by Hanson ('19 a), present as cartilaginous nodules in the embryonic shoulder-girdle. Similar bones have been described for other animals by several of the early workers in comparative anatomy (Parker, '68; Flower, '85, and Gegenbaur, '98).

Several names have been applied to these bony elements. Parker designated them as omosterna, while Gegenbaur referred to them as praeclavia. Since it is my opinion that these structures become more closely associated in the higher vertebrates with the clavicle than with the sternum, Gegenbaur's nomenclature will be followed.

The praeclavia are present as well-defined bones in rats six months of age or older. The shape of the bones is that of a truncated cone. They are symmetrically placed, and their bases are in contact with the sternal ends of the clavicles, while the bodies of the bones lie in two shallow fossae on the dorsal surface of the manubrium sterni (fig. 1). No articular cavities are developed at either end of the praeclavia; still the union of clavicle, praeclavium, and manubrium is not a very rigid one. A thin capsule extends from the sternal end of the clavicle to the dorsal surface of the manubrium, forming a complete investment for the small intervening bone. The line of attachment of the capsule to the clavicle may be easily mistaken for an epiphyseal line.

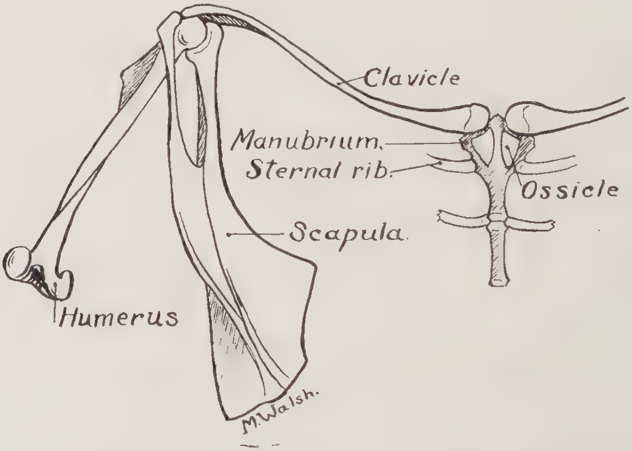

Fig. 1 Outline drawing of a dorsal view of the shoulder-girdle, showing the position of the praeclavia in relation to the sternum and clavicles.

In rats one and two months old the praeclavia are present as small cartilaginous nodules. The first ossification center (fig. 2) appears at the end of the third month after birth and ossification progresses rapidly. At the end of the sixth month, the bone has attained practically its mature size and form.

The ossification of the clavicle has been studied by Strong ('25). He did not observe any epiphyseal center of ossification for the sternal end of the clavicle, and the writer, in both cleared and sectioned material, has also failed to find any trace of an epiphysis for the clavicle.

Stevenson ('24), in a study of epiphyseal union in man, found a remarkable constancy in the order in which the epiphyses of any individual unite. Although the process of epiphyseal union may be slightly accelerated or retarded, yet, when the process has once begun, the sequence of union among the various epiphyses is observed to be exactly the same in every individual (p. 89). In the rat (Dawson, '24) the order of epiphyseal union is found to parallel to a remarkable extent the order that Stevenson noted in man. In man the period of epiphyseal union occupies only a small percentage of the total life-span, beginning in the fifteenth year and ending at the age of twenty-eight (Stevenson, '24). In the rat epiphyseal union begins in the fourth week after birth and seems hardly to be completed even in senility. In

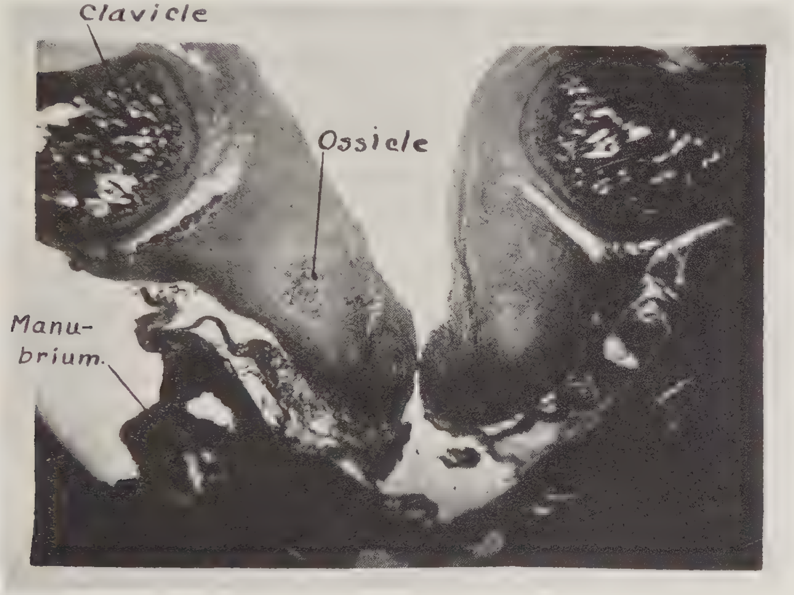

Fig. 2 Photograph of a frontal section of the anterior portion of the shoulder-girdle. The upper portion of the manubrium does not appear in the picture, but the relation of the clavicle and praeclavium is shown. Note the small ossification center in the praeclavium and the absence of any trace of an epiphysis in the clavicle. Age of rat, ninety-two days.

man the sternal epiphysis of the clavicle appears in the seventeenth or eighteenth year and finally unites in the twenty-eighth year (Stevenson, '24). In the rat the ossification center of the praeclavium appears at the end of the third month and the bone has never been observed to unite with the clavicle. The time of appearance of the sternal epiphysis of the clavicle in man, in terms of the period of epiphyseal union, is in the age interval of union of the proximal epiphysis of the radius and the union of the distal epiphyses of tibia and fibula. Similarly, the appearance of the ossification center for the praeclavium in the rat occurs at relatively the same time in the period of epiphyseal union, i.e., precedes slightly the union of the epiphyses of the distal ends of the tibia and fibula.

The two facts presented above, 1) the absence of a sternal epiphysis in the clavicle of the rat and, 2) the presence in this animal at the sternal end of the clavicle of a small bone whose ossification history parallels closely that of the sternal epiphysis of the human clavicle, form the basis for the suggestion made in the opening paragraph, viz., that the praeclavium of the rat may be homologized with the sternal epiphysis of the clavicle in man.

The conclusions of embryologists and comparative anatomists also tend to strengthen this view. The sternum of mammals is now regarded as a derivative of the shoulder-girdle (Hanson, '19 b), and in the earlier developmental phases it is continuous with the procoracoid element in the ventral end of the clavicle (Keith, '21).

Huntington ('19) and Keith ('21) support the theory of the phylogenetically double origin of the clavicle, a procoracoid overlaid by a dermal element. In the embryo this double origin is not always apparent, since the membrane bone develops from the mesenchyme before the cartilage is differentiated, but cartilage eventually appears at both ends of the clavicle. The congenital defect craniocleidodysostosis also supports the theory of a double origin. In this anomaly all the bones of membrane origin are poorly developed or lacking, but the clavicle is usually represented by a sternal and acromial element united by a ligament.

Huntington definitely homologizes the sternal epiphysis of the clavicle with the ventral end of the procoracoid (p. 383). In the rat, therefore, when the mesenchymatous shoulder-girdle becomes segmented into its component parts, the element representing the praeclavium is separated from the procoracoid core of the clavicle and remains separated through life. Very much the same thing occurs in the sternum in this animal, i.e., the sternebrae ossify from separate elements and remain separate. On the other hand, in man the segmentation of the sternum for the most part is lost. Similarly, in man the element represented by the praeclavium in the rat is not separated from the clavicle and in its ossification appears as the sternal epiphysis. This homology, it is believed, explains the unique behavior of the sternal epiphysis in man, making its appearance long after all other epiphyses, and not uniting with the clavicle until the twenty-eighth year.

SUMMARY

The location and ossification of a small bone between the clavicle and sternum is described. Following Gegenbaur, this bone is called the praeclavium.

It is suggested that this bone in the rat is the homologue of the sternal epiphysis of the clavicle in man. Two facts support this view: 1) the absence of a sternal epiphysis in the clavicle of the rat and, 2) the presence of the praeclavium whose ossification history parallels closely that of the sternal epiphysis in man.

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SUR UNE FORME PARTICULIÉRE DE TRANSFORMATION FOLLICULAIRE CARACTÉRISTIQUE DE L'OVAIRE DU LÉROT (ELIOMYS QUERCINUS, L.): FAUX CORPS JAUNES MÉTAPLASTIQUES

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NEUF FIGURES

Dans une note sur l'atrésie comparée de l'épithélium folliculaire chez les mammifères(1), nous avons signalé en passant un type de transformation folliculaire spéciale que nous avons trouvé dans l'ovaire du Lérot.

En effet, on y constate deux types d'évolution folliculaire: *a*) un premier type suit la marche atrétique commune, chromatolytique(2) et porte à la formation de faux corps jaunes; elle se fait remarquer par quelques détails dont nous nous occuperons plus tard; *b*) un deuxième type présente des modalités tout à fait spéciales: les éléments de la granulosa se transforment directement, par métaplasie, en des cellules d'un type spécial dont l'évolution rappelle les éléments interstitiels vulgaires.

Examinant la figure 1, nous voyons épars dans toute l'organe des nodules en fer à cheval (de beaucoup les plus fréquentes), des nodules circulaires, etc. (fig. 1; fig. 2, *B* e *C;* fig. 3, *b* e *c*). Ces nodules, que nous appellerons *faux corps jaunes métaplastiques,* sont constitués par un hile vasculaire, entouré de cellules à cytoplasme vacuolisé ou granuleux représentées dans les figures 5 et 6 et d'un cortex en fer à cheval constitué par des cellules sombres, à cytoplasme condensé (fig. 4). Dans les nodules circulaires le hile est central en rapport avec des cellules du type représenté sur les figures

5 et 6, le tout cerclé par des cellules ayant l'aspect qu'on voit dans la figure 4.

Après résorption des septa conjonctifs ou par un autre processus, ce que nous n'avons encore pu établir, ces blocs confluent et forment de grandes nodules (fig. 1, fig. 2, *A;* fig. 3, *A*).

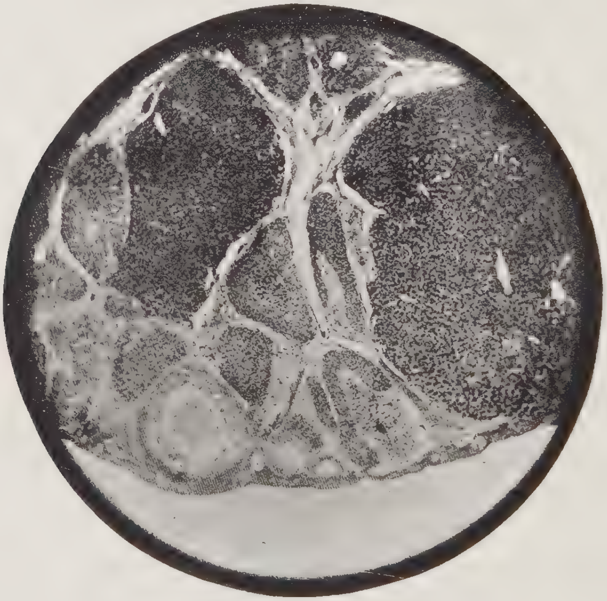

Fig. 1 Coupe de l'ovaire du Lérot.[1]

Ces grandes nodules sont formés par les éléments dessinés dans les figures 5, 6 et 7, qui se disposent en colonnes, rappelant les cordons de Remak; parmi ceux-ci circulent des éléments du sang. Ici et là on voit des grands capillaires en communication avec les canalicules intercellulaires. Dans son ensemble, le tout présente, quelquefois, un aspect hépatiforme.

Il faut dire que l'évolution et la constitution cytologique permet établir un diagnostique diférenciel sûr entre ces grands nodules et les vrais corps jaunes.

---

[1] Dessins de l'auteur; microphotographies de A. Costa.

En étudiant ces différents formations (faux corps jaunes métaplastiques et grand nodule métaplastique) nous constatons que leurs éléments constituitifs évoluent de cette facon:

En examinant, par exemple, un nodule en fer à cheval, nous voyons dans la partie encore avasculaire des nodules, des cellules à noyau excentrique, à cytoplasme condensé, prenant avidement l'éosine; acidophilie à part, cet élément présente l'aspect d'une plasmazellen (fig. 4).

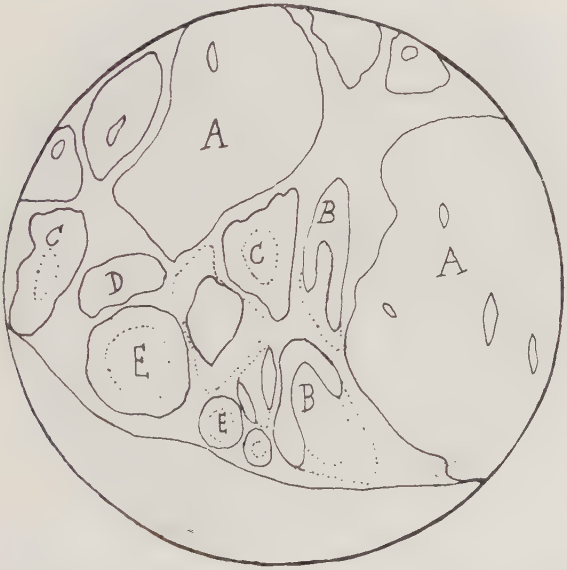

Fig. 2 Diagramme de la figure 1. *A,* grand nodule métaplastique; *B,* nodule en fer à cheval; *C,* nodule circulaire; *D,* nodule interstitiel métaplastique; *E,* follicules de De Graaf en voie d'atrésie chromatolytique. Zenker-hematox. ferr.-éosine. Microphotographie: Oc. 3, obj. 6, Leitz-Wetzlar.

Au fur et à mesure que ces éléments entrent en contact avec le sang, ils montrent des changements, qu'on peut résumer ainsi; *a*) Le noyau d'excentrique qu'il était, devient central, il se colore plus intensivement par l'hématoxiline; le nombre des caryosomes est plus grand. Le cytoplasme grandit peu à peu; l'éosine retenue d'abord intensivement, disparaît et sous peu il ne reste que des taches éosinophiles dans le cytoplasme (fig. 5). *b*) Ensuite il commence à apparaître dans le cytoplasme des granulations sidérophiles, et, d'une facon graduelle, l'élément tout entier devient granuleux. *c*) Le cytoplasme se peuple des vacuoles, d'abord petits, qui plus tard grandissent et préparent la dernière étape cytologique. Ce sont fréquemment des cellules binuclées, qui ont l'aspect de certains éléments de l'hypophyse appelés grands siderophiles

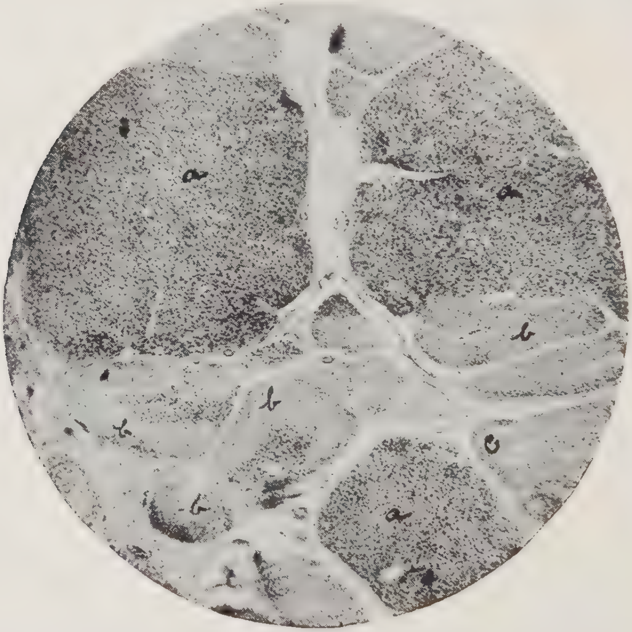

Fig. 3 Coupe de l'ovaire du Lérot. Il montre un aspect identique au de la coupe representée dans la figure 1. *a*, grand nodule métaplastique; *b*, faux corps jaune métaplastique (nodule en fer à cheval); *c*, faux corps jaune métaplastique (nodule circulaire). Zenker-haematox-ferr.-éosine. Microphotographie: Oc. 3, obj. 6, Leitz-Wetzlar.

(Soyer) (fig. 6). *d*) La dernière étape cytologique est représentée par la figure 7; ce sont des éléments du type interstitiel à cytoplasme vacuolisé, etc. Leur volume est plus grand que dans les cellules issues de la métaplasie d'emblée, qu'on observe en d'autres formations decrites plus loin.

À côté des formations que nous venons de décrire, nous voyons une autre espèce, formés de cellules d'aspect interstitiel, ayant la forme habituelle du follicule de De Graaf (ovoids, ronds, etc.) et dont la grandeur varie entre les limites d'un petit follicule et d'un follicule moyen (fig. 1; fig. 2, *D;* fig. 8). Ce nodule que nous designons sous le nom de *nodule interstitiel métaplastique* est constitué par des éléments polygonaux, à noyau central, avec un caryosome très net; leur cytoplasme clair et criblé de vacuoles a une structure alvéolairs et est bien delimité par la membrane cellulaire. La grandeur de ces éléments est, au début de ce processus métaplastique, un peu plus petite que celle des éléments interstitiels vulgaires (figs. 8 et 9). En examinant plusieurs de ces nodules nous voyons que leurs différents aspects morphologiques traduisent l'extension du processus métaplastique. En effet, dans un nodule, nous constators des débris de l'oocyte et de la pellucide environnés par une petite nappe de cellules métaplasiées; dans un autre on ne voit plus qu'une nappe de cellules interstitielles, substituant les éléments folliculaires, nappe toujours croissante (fig. 2, *D;* figs. 8 et 9). Nous devons dire,

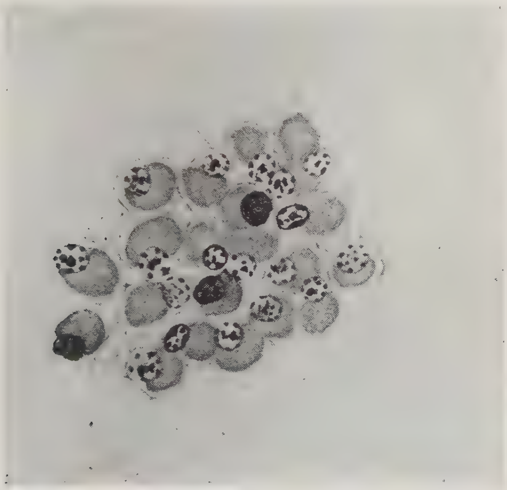

Fig. 4 Faux corps jaune métaplastique (nodule en fer à cheval). Cellules à noyau excentrique, grains chromatiques à la périphérie; cytoplasme homogène éosinophile. Zenker-hematox. ferr.-éosine. Dessin à la chambre claire Leitz-Wetzlar; obj. imm. 1/12. Table à dessiner inclinée de 45°.

cependant, que tous les éléments n'éprouvent pas cette métaplasie. Il y a des cellules qui évoluent dans le sens thécal et quelques unes même prennent l'aspect endothélial; nous verrons plus tard la signification de ce phénomène.

Ces éléments sont séparés par des cellules conjonctives, formant, quelquefois, des tractus conjonctifs, qui commandent la formation et pénétration des vaisseaux. En effet, nous assistons, dans une phase plus avancée, à l'invasion du nodule par des formations vasculaires. Parmi ces éléments on voit circuler des hématies, et, il faut le remarquer, l'apparition des éléments du sang est très précoce; on y voit quelques uns dans un moment ou il est assez difficile de dire si la membrane de Slawjansky est rompue.

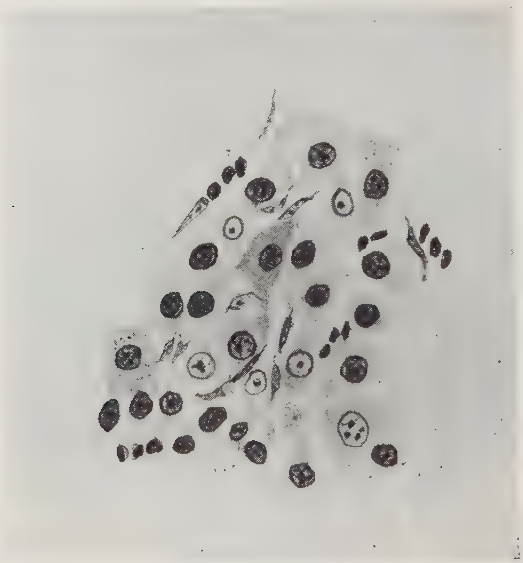

Fig. 5 Faux corps jaune métaplastique (nodule en fer à cheval). Cellules polygonales à noyau central; cytoplasme semé de taches éosinophiles. Quelques éléments ont des grains sidérophiles. Zenker-hematox. ferr.-éosine. Dessin à la chambre claire Leitz-Wetzlar; obj. imm. 1/12.

En resumée: la métaplasie des éléments de la granulosa en des cellules du type interstitiel présente, par conséquence,

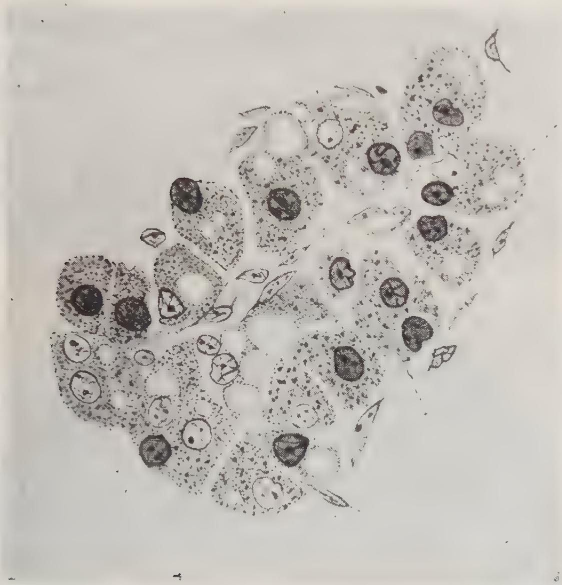

Figure 6

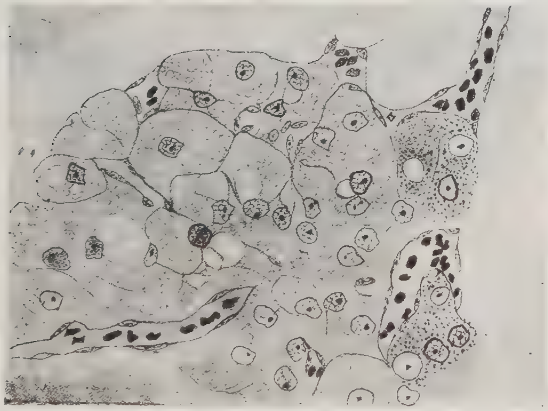

Figure 7

Fig. 6 Grand nodule métaplastique. Cellules à noyau fortement coloré par l'hematox. ferr.; quelques cellules sont binuclées; cytoplasme vacuolisé et parsemé de granulations sidérophiles. Zenker-hematox. ferr.-éosine. Dessin à la chambre claire Leitz-Wetzlar; obj. imm. 1/12. Table à dessiner inclinée de 45°.

Fig. 7 Grand nodule métaplastique. Cellules du type interstitiel. On voit des éléments granuleux autour des capillaires. Zenker-hematox. ferr.-éosine. Dessin à la chambre claire Leitz-Wetzlar; obj. imm. 1/12. Table à dessiner inclinée de 45°.

deux types d'évolution: *a*) ou la métaplasie se fait lentement et les follicules se transforment en des *faux corps jaunes métaplastiques* qui confluent, il semble, et forment le *grand nodules métaplastique* (homologue de la glande interstitielle?); en ce cas les éléments de l'épithélium folliculaire traversent des différents stades (figs. 4, 5, 6) jusqu'au type de la cellule interstitielle adulte (fig. 7); *b*) ou bien le processus histogenètique se déclanche rapidement, la métaplasie est brusque, les éléments de la granulosa prennent d'emblée l'aspect interstitiel et le follicule se transforme dans un *nodule interstitiel métaplastique* (figs. 8 et 9). On peut dire que ce qui caractèrise, essentielment, ce type c'est la rapidité d'évolution cytologique par rapport aux éléments des faux corps jaunes métaplastiques: leurs éléments atteignent d'emblée l'état adulte.

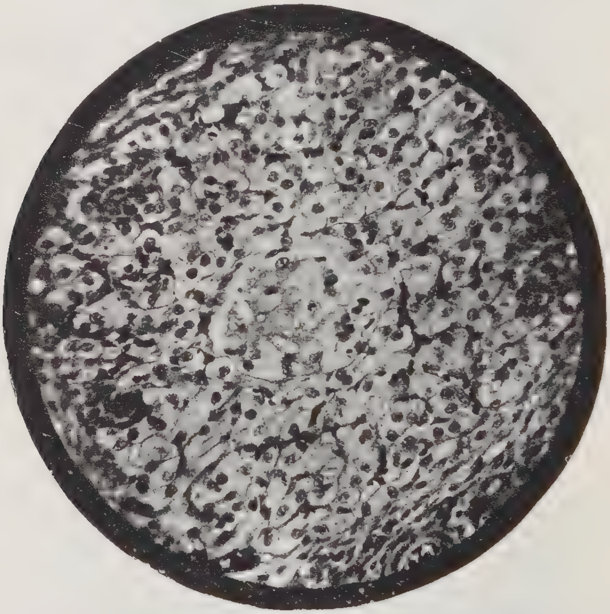

Fig. 8 Nodule interstitiel métaplastique. Ce nodule, issu de la métaplasie immediate de la granulosa en des cellules du type interstitiel, présente encore au centre des débris de l'oocyte. Bouin-hematox. ferr.-éosine. Microphotographie: Oc. 3, obj. 6, Leitz-Wetzlar.

L'histogénèse de ces formations métaplastiques du Lérot est très complexe. D'ores et déjà nous dirons seulement que cette transformation est determinée par des processus de vascularisation systématique du follicule, processus présentant des caractèristiques tout à fait spéciales, que nous décrirons dans une autre note.

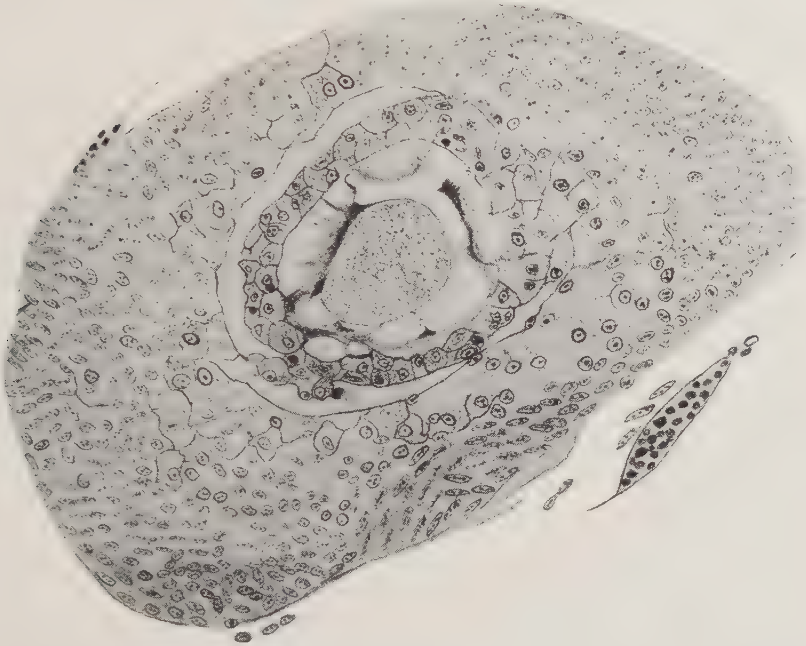

Fig. 9 Follicule de De Graaf. Début de la transformation métaplastique: on voit encore nettement figuré l'oocyte; les éléments de la corona radiata et du cumulus proligère sont déjà métaplasiés. Bouin-hematox. ferr.-éosine. Dessin à la chambre claire Leitz-Wetzlar, obj. 7. Table à dessiner inclinée de 45°.

Des histologistes ont déjà signalé des transformations folliculaires aprés la pénétration des vaisseaux dans les follicules normaux ou atrétiques: Wagener, Schulin, Créty, Rabl, (cités par Athias, Winiwarter et Sainmont), Van Beneden(4), Athias(5), Winiwarter et Sainmont(6). Dans les cas publiés par ces auteurs il s'agit de phénomènes tout à fait sporadiques, tandis que chez le Lérot la métaplasie qui vient d'être décrite présente un caractère singulièrement systématique.

C'est dans le matériel que M. le Prof. Athias avait bien voulu nous prêter pour notre étude sur l'atrésie comparée (C. R. Soc. Biol., t. 88., 1923) que nous avons trouvé la métaplasie folliculaire qui vient d'être sommairement décrite; M. Athias lui-même nous a conseillé de poursuivre l'étude en question, en nous cédant gracieusement son matériel: nous le remercions ici vivement.

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SOME REMARKS ON THE POLYOESTRUS OF PRIMATES

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ONE FIGURE

In connection with the series of important articles in The American Journal of Anatomy, vol. 32, no. 3, on the oestrous cycle of mammals, it will perhaps be of some interest to add the following note:

In the year 1905, while studying the menstrual changes in the uteri of Cercocebus cynomolgus in the laboratory of the late Professor Hubrecht at the University of Utrecht, I had at the same time at my disposal a large number of pregnant and non-pregnant uteri of Tarsius spectrum, which enabled me to prove the existence of a polyoestrus in this primitive Primate. The material of more than 1000 uteri and ovaries collected by Hubrecht in the Isle of Banka (East Indies) had been catalogued with accurate data of fixation, etc., during several years. It appeared that in every month of the year pregnant females had been killed, the most of them in October and November at the end of the dry season in this island. The pregnancy curve (here reproduced in fig. 1) follows the same line, when only the very young stages of pregnancy, before the closure of the amnion, are considered; so one may accept the fact that the duration of pregnancy in Tarsius cannot be very long. Putting the maximum of the curve a bit to the left, the pregnancy curve may be considered as the conception curve during the different months of the year. Among the non-pregnant uteri of Tarsius there were found several menstruating ones with swollen hyperaemic mucosa and extravasation, but without the destruction of the uterine tissue which I have found (as also did Heape) in monkeys.

The investigation of Tarsius and Cercocebus led me to the conclusion that in Primates (among whom Tarsius spectrum takes a very primitive place) polyoestrus with a continual capacity of conception must be considered as the primitive state. A continual series of ovarian cycles is accompanied by continual periodic changes in the mucosa uteri. The manner in which the uteri of Tarsius and Cercocebus react in every

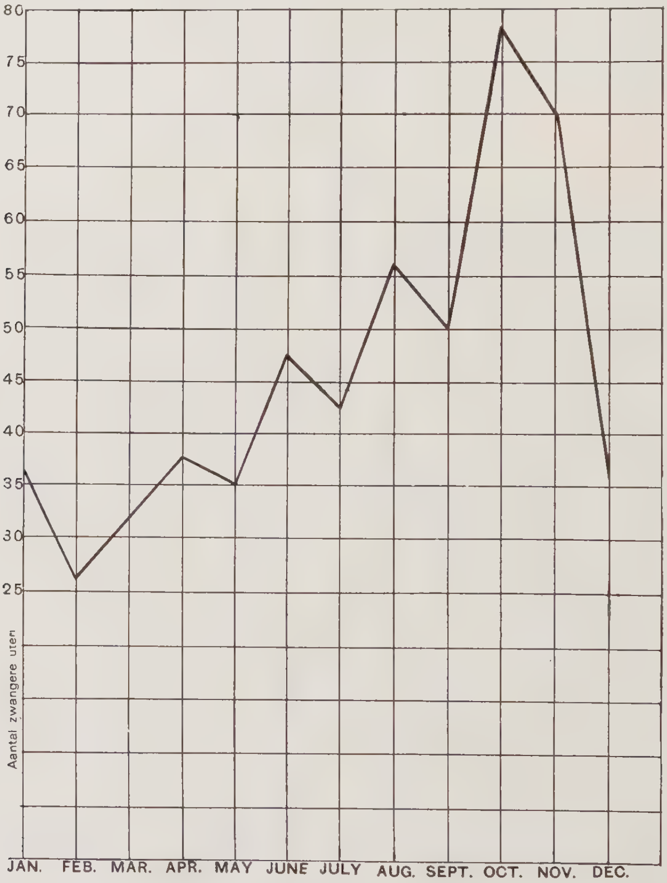

Figure 1

oestrus (for instance, the localization of the uterine bleeding and prooestrum at the places where the placenta will be formed in pregnancy) has led me to consider these changes as originally caused by a developing ovum whose trophoblast irritated the uterine wall—a function taken over in later times by the ovum in the ovary itself before reaching or without reaching the uterus. According to this conception, the uterine changes originally formed a part of the process of placentation.

It may be that the inclination to a higher productivity in special oestrous cycles, as shown by Tarsius, has led in further phylogenetic stages to a state such as Heape found in Semnopithecus and Macacus and to which also my Cercocebus material points, that is, to the restriction of the power of conception to special periods, with the conservation of the remaining menstrual cycles, which may be considered as the results of the primary sexual conditions. Thus there exist in Cercocebus menstrual cycles without ovulation, during which the uterine changes are much less accentuated than during menstruation when accompanied by ripe ovarian follicles or corpora lutea. So it may be possible that there are special periods of conceptibility in these monkeys in the state of nature. As in man, the uninterrupted menstrual cycles in monkeys may be a direct continuation of the polyoestrus of primitive ancestors. In man, also, there are indications of a sexual periodicity, as I have previously stated—as have other investigators before me—from the study of birth statistics in several countries.

The results of the researches here indicated have been published only in the Dutch language in my dissertation: Bydrage tot de kennis van Menstrueelen Cyclus en Puerperium. Utrecht, 1905. A summary is to be found in Monatschrift für Geburtshülfe und Gynaecologie, vol. 24, p. 730.

QUANTITATIVE STUDIES OF THE TESTIS

I. SOME OBSERVATIONS ON THE CRYPTORCHID TESTES OF SHEEP AND SWINE

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TWENTY-TWO FIGURES

"A systematic numerical study of the anatomy of the endocrine system is thus a necessary condition for an exact treatment of the important problems that are to be solved in this field. . . . What is our present knowledge of the extent to which the so-called gland of puberty is to be found at a certain age in a normal human sex gland?" These sentences occur in a paper by Hammar.

Rasmussen, in his study of the human hypophysis, has described in detail a quantitative method, reporting that a similar one has also been used by Godlewski, Hammar, Jackson, and others.

The need for the use of such a method in the study of the testis is apparent to any one familiar with the literature. Despite the absence of such quantitative study, there has been no lack of expressed opinions as to the function of the interstitial cells of the testis and also as to their hypertrophy or non-hypertrophy under various conditions. A few cases, selected from other similar ones, will make this more apparent.[1]

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[1] Since the following study was made, no. 2 of volume 34 of The American Journal of Anatomy, containing the papers of Moore, Moore and Quick, and Nonidez, has appeared. In so far as they relate to interstitial cells, these papers are not definitely quantitative. Moore has planned and executed experiments designed to show hypertrophy or its absence with regard to interstitial cells, but, while the weights of the testes have been carefully recorded, judgments as to amounts of interstitial cells present have been formed, as by previous workers, from microscopic study and not by a quantitative method.

Thorek stated, "The Leydig cells are alone responsible for the production of the internal secretion that gives rise to the secondary sex-characters, sexual potency and cerebral eroticising products." This very definite statement was reported as based upon a series of six experiments on monkeys, observations upon patients, three of whom were described in some detail, and upon the findings of other investigators, among whom may be mentioned Bouin and Ancel, Hanes, and Massaglia. Thorek, in describing the figures illustrating the microscopic findings in these three patients, uses the phrases, "Note . . . . scantiness of interstitial tissue" and "Interstitium increased in quantity," but gives no definite data as to the size of the testes involved beyond recording that in one case the testes were smaller than normal.

Massaglia, in setting forth the results of his experiments on roosters, states that (case VII) in the left testis weighing 11 grams "the interstitial connective tissue was increased; the Leydig's cells were normal in number," while in the right testis, weighing 2.5 grams, "there was a marked increase of the interstitial connective tissue and increase of the number of the Leydig's cells." Whether the increase was believed to be relative or absolute was not specified. Apparently it was deduced from microscopic examination only.

Hanes studied "fifty-six cryptorchid testes from the pig, seven from the sheep and six from man." He states, "*A compensatory hypertrophy*[2] can be demonstrated in the interstitial cells under certain conditions. In a pig from which one testicle has been removed at an early age, the interstitial cells of the remaining organ are present in markedly increased numbers. So striking is this increase that one can easily tell microscopically the monoscrotal from the biscrotal organ. (Bouin and Ancel, '03)." In all his discussion of this subject he does not mention the weights of the testes which he studied.

Bouin and Ancel recorded in their papers the weights of some of the pig testes which they studied, but seem to have made judgments as to relative quantities of interstitial cells present solely on the basis of microscopic examination.

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[2] The italics are Hanes'.

Oslund, in treating this subject in rats and Guinea pigs, states ''no compensatory hypertrophy was anywhere seen,'' but does not record the weights of testes studied nor does he give the method whereby hypertrophy or its absence was determined.

The following paper sets forth what is, so far as I know, the first attempt to apply a quantitative method to the study of the elements of the testis. I wish to thank Dr. W. H. F. Addison and Dr. H. L. Osterud for helpful suggestions. Thanks are also due to Dr. W. R. Andress, Dr. A. Spitz, Mr. P. H. Brunner, and Mr. Alexander Anderson for their kindness and assistance in securing material.

MATERIAL AND METHODS

The series of testes used in this study was collected in abattoirs in Philadelphia. Absolute data as to the ages of the animals from which testes were taken cannot be given, but the probability is that it was about six to nine months for most of the pigs, and the same for the sheep. Each testis was cleaned of epididymis, fat, and other extraneous tissue and weighed. A block of tissue was removed from the center of the convex surface, opposite to the epididymis, fixed in Bouin's, sectioned in paraffin, and stained with Delafield's hematoxylin and eosin or with the hematoxylin alone, and mounted in balsam.

Such sections were studied microscopically. A drawing of a representative field from each pig testis and from several sheep testes were made with an Edinger projection apparatus. Tracings of the sketches of pig testes were cut out with scissors, tubular areas and interstitial areas being weighed separately, and from these weights percentages were calculated. The weights of these testes being known, weights of interstitial tissue and of tubules could be calculated for each testis. The pattern of the pig's testis is especially fitted for such study, as will be pointed out later. The results of the above calculations are given in table 2.

This method was tested in the following way. From six pig testes taken at random from the series, sections from other parts of the testes were made and compared with the first sections already mentioned. This study indicated that the pattern of a given testis was essentially uniform throughout. Sample sections of both testes of a given animal, where the two corresponded closely in weight, gave results almost identical (table 2). This also indicates that the method gives reliable results when used in studying the pig testis.

Microscopic examination of sheep material showed interstitial cells to be extremely scanty in both normals and, with one or two exceptions, in cryptorchids, and so a quantitative study was not undertaken.

DISCUSSION

It is obvious that before determining quantitatively the hypertrophy or the atrophy of any element of any organ the weight of the organ itself must be considered. This was overlooked entirely by Hanes, Thorek, and Oslund, although weights of testes were recorded by Massaglia and in part by Bouin and Ancel. An examination of table 1 shows at once that weights alone do not indicate compensatory hypertrophy of the cryptorchid testis in sheep when the other testis has been removed. An examination of the weights of the testes of pigs listed in table 2 shows that hypertrophy is not the rule for the cryptorchid testes of semicastrated swine. Hatai, working on rats, reported that testes removed subsequent to semicastration showed a gain of from 6 to 25 per cent in weight, the average being 14 per cent. No such conclusion can be drawn from noting weights in the above series.

Turning now from the question of the weights of testes to a microscopic study of this material, even the most cursory microscopical examination of normal pig and sheep testes will reveal a striking difference in their pattern. This was noted by Bouin and Ancel, who reported that interstitial cells are much fewer in the dog, bull, or ram than in the horse or swine, relatively rare in man, and even more so in the rodents.

This scarcity of interstitial cells in the sheep testis has also been noted by Moore and Oslund.

Bouin and Ancel and Hanes studied the cryptorchid testes of both sheep and swine. The former state that the cryptorchid testes of adults of both species offer essentially the same structure. Hanes records, as do Bouin and Ancel, that the cryptorchid testes of sheep show no spermatozoa, but beyond that he ignores them in drawing a positive conclusion

TABLE 1

Weights of sheep testes

CASE NO.	WEIGHT OF EACH TESTIS	CASE NO.	WEIGHT OF EACH TESTIS
Bilateral cryptorchids			
	grams		*grams*
M276	15	M184	30
M231	16.5	M225	42.5
M278	20	M275 A	45.5
M230	22	M275 B	1.8
M287	22	(epididymis only present)	
M161	24	M224	48
M277	25		
Monolateral cryptorchids			
M167	1.5	M287	25
M294	3.6	M296	26
M279	4.4	M267	31.3
M163	5	M226	33
M164	5.5	M121	35
M295	6	M271	35.5
M169	6	M240	36.5
M265	11	M205	43
M204	11.5	M185	44
M171	11.5	M287	45
M286	14	M168	52
M162	14.5	M223	53
M143	19	M216	54
M158	21	M122	61
Normal animal			
M170	166, adult.		

TABLE 2

Testes of swine

CASE NO.	WEIGHT OF TESTIS	INTERSTITIAL CELLS		TUBULES	
		Bilateral cryptorchids			
	grams	*per cent*	*grams*	*per cent*	*grams*
M281 A	41	54.54	22.36	45.46	18.64
B	44.5	54.23	24.13	45.77	20.37
M234 A*	45	38.02	17.11	60.01	27
B	46	38.52	17.72	61.48	28.28
M270 A	55	68.5	37.68	31.5	17.32
B*	39.5	45.77	18.08	45.26	17.88
M273 A	46.5	50.2	23.34	49.8	23.16
B	52	52.3	27.19	47.7	24.8
		Monolateral cryptorchids			
M221	14.5	70.4	10.2	29.6	4.29
M219	17	45.2	7.68	54.8	9.31
M268	28.5	39.32	11.21	60.68	17.29
M214*	30	55.74	16.72	37.62	11.28
M242	34	21.26	7.23	78.74	26.77
M233	35.5	59.83	21.24	40.17	14.26
M186	36	54.54	19.64	45.46	16.36
M263*	36.5	43.79	16	51.55	18.81
M235	37.5	46.53	17.45	53.47	20.05
M251	40	50.03	20	49.97	19.99
M262	40	44.8	17.92	55.2	22.08
M274	40	53.24	21.3	46.76	18.7
M252	44.5	73.2	32.57	26.8	11.43
M241*	45	58.69	26.41	39.13	17.61
M248	49	37.46	18.25	62.54	30.65
M239	51.5	47.79	24.61	52.21	26.89
M237	55	49.83	27.41	50.17	27.59
M218	56	61.17	34.26	38.83	21.74
M238*	57.5	41.69	23.97	57.27	32.93
M232	58.5	38.06	22.27	61.94	36.23
M282	60.5	65.9	39.87	34.1	20.63
M213	64	74.78	47.86	25.22	16.14
M236	64	48.41	30.98	51.59	33.02
M269	71.5	53.69	38.39	46.31	33.11
M264	72	67.43	48.45	32.57	23.55
M253	74.5	63.01	46.94	36.99	27.56
M247	75	56.96	42.72	43.04	32.28
M250	78	41.82	32.62	58.18	45.38
M249	79.5	39.06	31.07	60.94	48.43
M217	80	71.55	57.24	28.45	22.76
M222*	88	64.45	56.72	33.14	29.16
M246	96	35.56	34.14	64.44	61.86
M243	172	47.63	81.92	52.37	90.08
M220	226	31.90	72.1	68.1	153.9
		Normal swine			
M284 A	218	36.8	80.22	63.2	137.77
M283	367	28.54	104.74	71.46	262.26

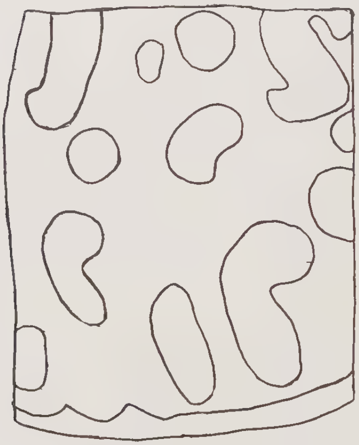
as to compensatory hypertrophy of the interstitial cells of the testis.

A study of the sheep testes listed in table 1 indicates that contrary to the statement of Bouin and Ancel, the cryptorchid testes of sheep and swine do not offer essentially the same structure, but those of sheep differ from those of swine in the same way as do normal testes of these species, namely, in the relative amount of interstitial cells present. Such study also indicates that hypertrophy of the interstitial cells of the testis does not occur in sheep following monolateral castration and that hypertrophy of the interstitial cells does not follow degeneration of the seminiferous tubules in cryptorchid testes of sheep. The last conclusion was also reached by Moore and Oslund.

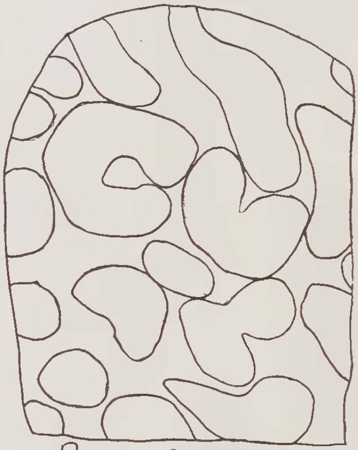
Taking up the series of pig testes from the microscopic standpoint, it is undoubtedly true that the pattern of these testes is extremely variable from one individual to another. Some testes have the tubules close together and others have wide spaces between the tubules, but in any case the spaces are almost or entirely filled with interstitial cells. It is this fact which makes the pig testis an especially favorable material for the study of interstitial cells, especially from the quantitative point of view. See figures 12 and 13 after Hanes, A and B after Bouin and Ancel, and my own figures (numbers corresponding to numbers of testes in table 2). However, a study of the data given in table 2 together with my figures shows how useless it is to consider microscopic pattern only, meanwhile neglecting weights of testes, in attempting to settle the question of the hypertrophy of the interstitial cells.

Compare first M221 and M213. Here are two testes of essentially the same pattern, yet the second contains nearly five times as much interstitial tissue as the first, simply because it weighs over four times as much. Again, take M219 and M246, two others of similar pattern. M246 has over four times as much interstitial tissue as M219 because, although its pattern is closer, it is over five times the weight of the latter.

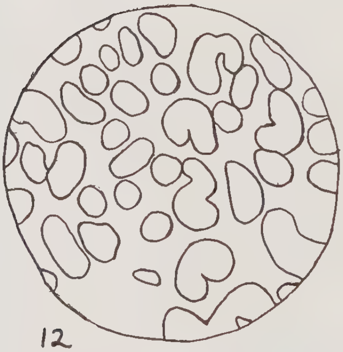
Changing the method of comparison, testes of similar weights may be selected. Compare M213 and M236, weighing 64 grams apiece. M213 has over 50 per cent more interstitial tissue than M236, in spite of their weights being equal.



A – 180 gms.



B – 80 gms



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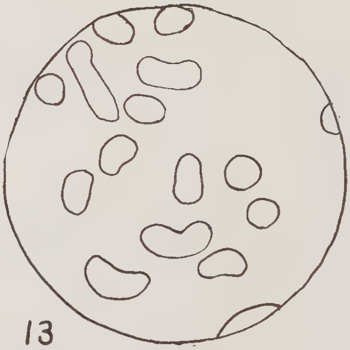

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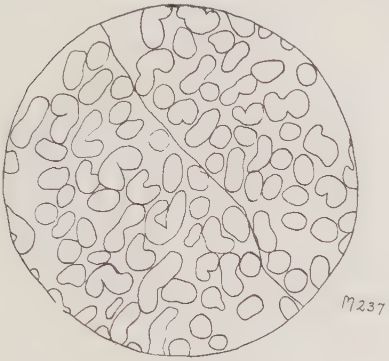
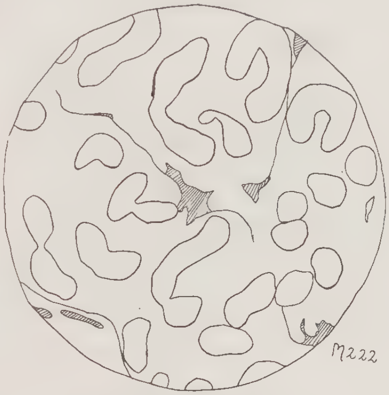
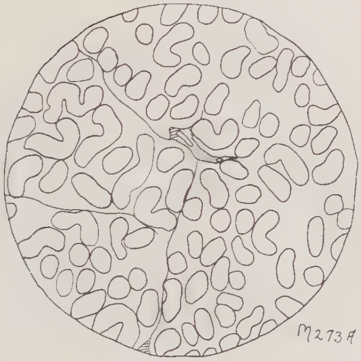
EXPLANATION OF FIGURES

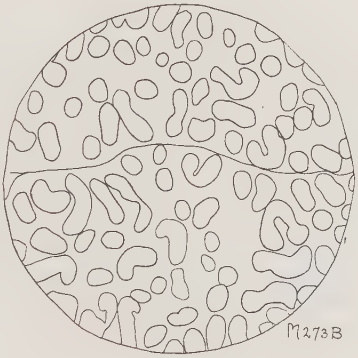
Figures A and B are traced from Bouin and Ancel ('03) and reduced one-half. Figures 12 and 13 are similarly traced from Hanes ('11). All other figures are from sections of pig testes in the series shown in table 2. They were drawn with an Edinger projection apparatus and as printed are magnified twenty-five diameters. Cross-hatched areas represent masses of fibrous connective tissue. The remainder of the intertubular spaces were filled with interstitial cells.

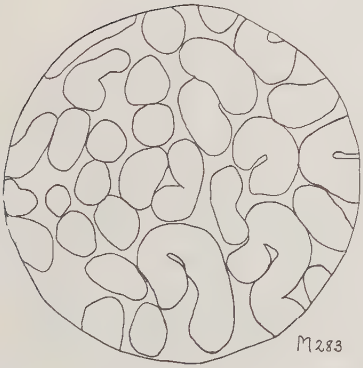
M270A and M237 also are of equal weights, 55 grams apiece, yet the former has 38 grams of interstitial tissue to only 27.5 grams in the latter. These examples are sufficient to demon-

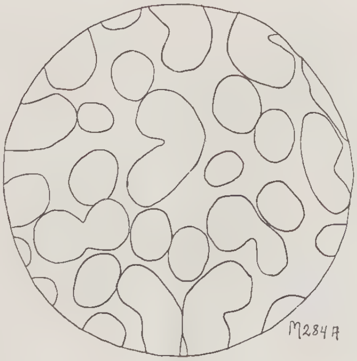






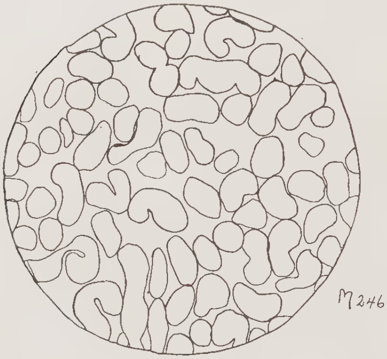


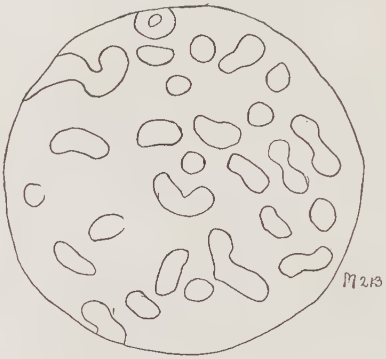


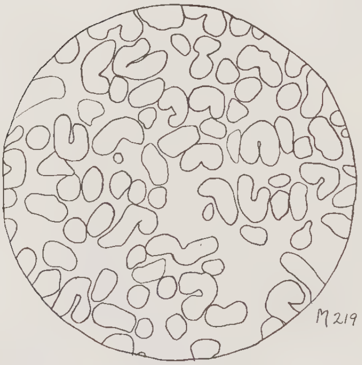


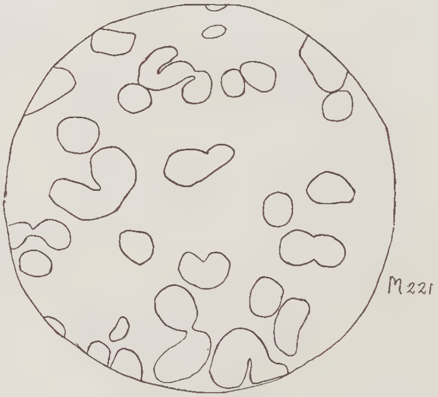
strate the possibilities for error in studying the testis when quantitative methods are not used.

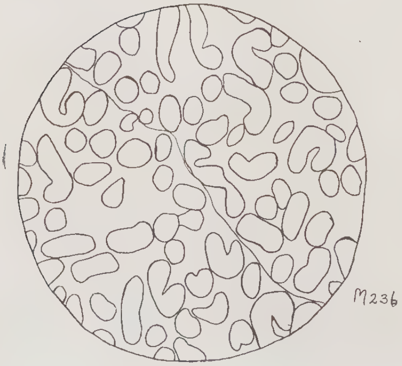
As was pointed out in the introduction, Bouin and Ancel and Hanes have paid particular attention to testes of swine

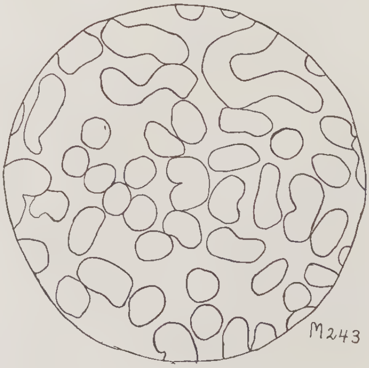




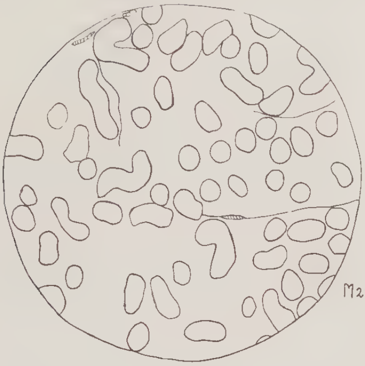


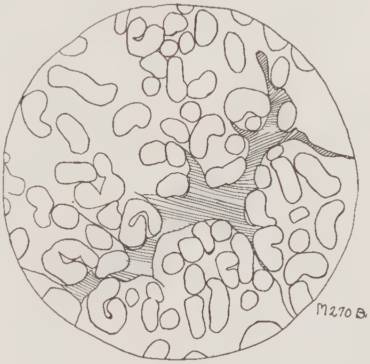


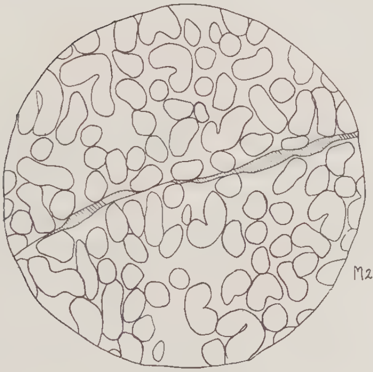


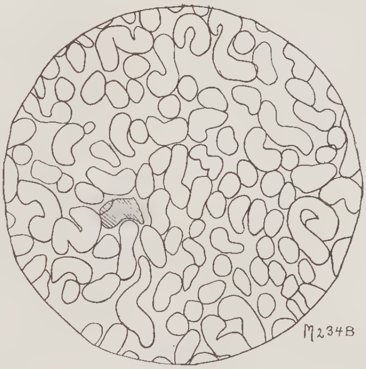


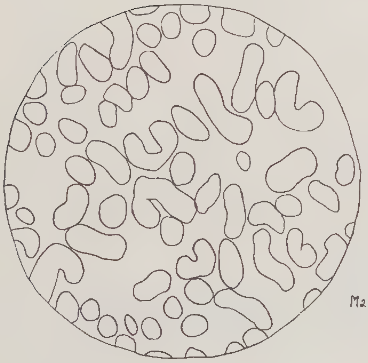
as illustrating compensatory hypertrophy of the interstitial cells. With this point in mind, an examination of table 2 and of my figures may again be made. A consideration of the varied patterns in figures M221, M219, M246, M213,

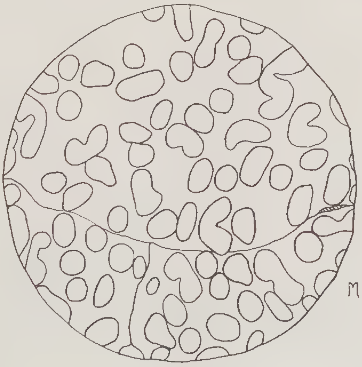

M270A


M270B


M234A


M234B


M281A


M281B

and M222, all figures being made from sections of testes from semicastrated, monolateral cryptorchid pigs, renders this idea very improbable. A comparison of the corresponding weights of interstitial tissue calculated for the same testes detracts still further from the probability of compensatory hypertrophy. A comparison of the figures taken from monolateral cryptorchids with those from bilaterals shows further that, contrary to Hanes' statement, it is not possible in many cases to distinguish such testes microscopically.

An analysis of cases M283 and M284 casts even more doubt on compensatory hypertrophy of the interstitial cells as well as refuting still further Hanes' statement as to the microscopic difference between monoscrotal and biscrotal testes. These animals were two boars, about one year old and weighing about 250 pounds each. Both were black and were so similar in size and general appearance that they might well have been brothers. When slaughtered, M283 was found to have one testicle congenitally absent. The other testicle was scrotal and weighed (less epididymis) 367 grams. Both testes were present in the scrotum of the other and weighed, respectively, 216 and 218 grams. A glance at figures M283 and M284A will show that the latter has the more open pattern, contrary to Hanes' statement. Calculations by the method already described showed that the single testis of M283 contained less than two-thirds as much interstitial tissue as those of M284. From this study of both normal and cryptorchid material, it seems well established that compensatory hypertrophy of the interstitial cells does not occur in swine as a result of semicastration.

It is interesting also to apply this quantitative method to some cases reported in the literature. It cannot be done in most instances because of lack of data accompanying illustrations and should not be too much insisted upon where possible, but at any rate it is interesting.

Bouin and Ancel published a figure from the testis of a monolateral cryptorchid and a figure from the testis of a bilateral cryptorchid as showing compensatory hypertrophy of the interstitial cells, and they record the weights of these testes as further proof of the same process. My figures a and b are tracings from the drawings published by the above workers. An application of the paper method shows the animal carrying but one testis had over 115 grams of interstitial tissue, while the other with both testes had less than 57 grams of interstitial tissue. This looks like hypertrophy, but it can hardly be called compensatory.

Oslund publishes figures 1, 2, 3, and 4, the first and third being from the scrotal right testes of rats and the second and fourth being from the cryptorchid left testes of the same two animals, the latter testes being about one-half the size of the former. He says nothing as to the interstitial tissue of the second rat, but concludes that "there was some hypertrophy of the interstitial tissue" in the cryptorchid testis of the first rat. An application of the paper method to these figures and a consideration of the fact that the cryptorchid testes were not more than one-half the size of the scrotal ones indicates that the cryptorchid testis of the second rat, in discussing which nothing was said concerning the quantity of the interstitial cells, had only about one-fourth as much interstitial tissue as the accompanying scrotal organ, while, in the case of the first rat where it was reported that "there was some hypertrophy of the interstitial tissue" in the cryptorchid testis, that testis, according to the fields figured and using this quantitative method, actually had less than one-half the amount of interstitial tissue occurring in the scrotal testis of the same animal. This confirms one result of a study of the series of pig testes listed in table 2, namely, microscopic study alone without the use of a quantitative method and a consideration of the weights of the testes involved may easily prove misleading as to hypertrophy or atrophy of the interstitial cells.

It seems profitable also to consider briefly some of the theories that have been advanced with regard to interstitial cells. The theories may be grouped arbitrarily as those having to do with the function of these cells and those having to do with the causes of their appearance or increase and decrease. A trophic function has been ascribed to these cells by Plato, Stieve, and Van Oordt. Bouin and Ancel, Hanes, Whitehead, Massaglia, Thorek, and others have believed that they have an endocrine function. The latter group, though not stating it in so many words, have apparently employed the assumption that the greater the volume of the interstitial 'gland' the greater must be its internal secretion. This assumption is not necessarily correct, and present knowledge in regard to the thyroid and its possible complications casts doubt upon it.

As I have pointed out elsewhere, either the trophic hypothesis or the endocrine hypothesis in regard to the interstitial cells of the testis seems to fit a selected group of facts, but neither hypothesis seems to have a universal application to the wide and well-established variations, both quantitative and qualitative, to which interstitial cells are subject.

Kingsbury, Humphrey, and Oslund have reported these cells as increasing coincidently with degenerative processes. The two former have regarded them as concomitants of such processes, but Humphrey reported that degenerative processes might occur without the accompaniment of interstitial cell hypertrophy. Observations already cited with regard to the sheep testis parallel this statement as does also Oslund's report.

Oslund has formulated a mechanistic hypothesis to account for hypertrophy or atrophy of interstitial cells, this hypothesis being also one of two suggested by Whitehead to account for observed changes in these cells in the testis of the pig embryo. "With degeneration of germinal epithelium, the seminiferous tubules decrease in size, thereby increasing the intertubular spaces. These spaces fill with lymph, bathing the connective tissue found therein and supplying it with abundant space and food for growth and proliferation. That this is what happens is shown by the fact that cells intergrading in structure between conective-tissue cells and interstitial cells are frequently found. The additional fact that the interstitial cells stain like the lymph in which they are bathed further supports this hypothesis. If the tubules again become active and enlarge, the pressure they exert upon these spongy, lipoid-filled cells causes them to decrease in volume or to atrophy, as was noted by Whitehead.''

If this theory is to have a wide application, the formation of wide spaces between the tubules should be always accompanied by hypertrophy of interstitial cells. Oslund has himself reported that degeneration may occur unaccompanied by hypertrophy of interstitial cells. Humphrey has reported the same thing, and this observation is confirmed with reference to the testis of the sheep. This limits the application of the theory in one direction. Is it then true that interstitial cells hypertrophy only in the event of degenerative processes? My observations on sheep and cattle embryos lead me to conclude that they may and do hypertrophy in the absence of degeneration. Rasmussen found the same to be true for the woodchuck and cites other instances. Finally, then, do interstitial cells atrophy because of pressure? Rasmussen states with regard to the woodchuck, ''In the spring as the animal is waking up from hibernation, the interstitial cells rapidly enlarge and apparently increase in number. . . . This great interstitial cell development forces apart the tubules and doubles the diameter of the testis . . . .''

Since no theory so far proposed fits all the facts, perhaps no theory is correct or possibly the significance of the interstitial cells of the testis varies in different species.

SUMMARY AND CONCLUSIONS

1. There is a great difference in the pattern of the testes of sheep and swine, whether normal or cryptorchid animals of the two species be compared. Ancel and Bouin noted the former fact, but apparently overlooked the latter. Hanes ignored both. This should be taken into account in evaluating their conclusions.

2. In neither species, according to my series of cases, does the remaining testicle, as a rule, hypertrophy after the removal of the other.

3. No rule of hypertrophy of the interstitial cells could be drawn from a microscopic study of the testes of sheep.

4. The testes of swine are especially fitted for a quantitative study of their interstitial cells.

5. The application of a quantitative method to this material indicates that Bouin and Ancel and Hanes were wrong in their conclusion that compensatory hypertrophy of the interstitial cells in swine follows semicastration. There is, judging from my material, no such law, or if there be such a law, its operation is modified by factors as yet undetermined.

6. This study shows the applicability of a quantitative method to the interstitial cells of the testis and emphasizes the necessity for the use of some such method, taking into account both weight and pattern of the testis, when attempting to prove or disprove the hypertrophy or atrophy of the interstitial cells.

7. Each of the theories so far advanced in regard to interstitial cells seems to fit accurately with a limited group of facts. No one of them fits all the facts. Hence perhaps it is safe to conclude for the present that none of these theories is correct or that possibly interstitial cells have a different significance in different species.

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ON THE LYMPHATIC SYSTEM OF THE NEWBORN RAT (MUS NORVEGICUS ALBINUS)

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FOUR FIGURES

A decade or two ago, interest hinged largely on the manner of the origin of the lymphatic system; did this arise as a continuous centrifugal growth from definitely established venous centers or as a result of a coalescence of independently formed mesenchymal spaces which later connected with the blood-vascular system? In this connection the question of the angioblastic origin of the intra-embryonic vascular system, advanced by His, which would preclude the possibility of an independently formed endothelial system, could not be sustained. Schulte, in 1914, and Stockard, in 1915, indicated that intra-embryonic endothelium may arise in situ from embryonic mesenchyme, and thus credit the plausibility of the independent origin of lymphatic endothelium. As regards the origin of the embryonic lymph sacs, early workers were largely in accord, but less so, perhaps, concerning their number. That these were of venous origin there could be no doubt; and all were agreed that the cervical pair arose by fenestration from the jugular venous channel as separate spaces, which later, by coalescence, formed an enlarged sac, the jugular lymph sac. At first distended with blood and completely cut off from its jugular origin, this sac secondarily acquired connections with the venous system at points which become the permanent communications between the two systems.

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In this study no attempt has been made to determine the origin of the lymphatic system in the white rat, valuable and interesting though it might be, but rather to determine the purely anatomic relations of the system in the young rat. The lymphatic system occupies a very important position in physiology, pathology, and clinical medicine, as is attested by its relation to tissue drainage, digestion, infection and inflammation, malignancy and edema. It is quite probable that the solution of many problems in medicine depend on an increase in the knowledge of the lymphatics. However, the increase in knowledge along this line depends considerably on the development of data concerning the anatomy and distribution of the entire system. Consequently, researches on the origin, development, method of growth, distribution, and anatomic relationships of the lymphatic system are of primary significance. The problem was first approached with the hope of determining the peripheral communications between the two vascular systems. Adult rats were first studied, and injections were made to delineate the lymph glands and channels, and to determine, if possible, the capillarial connections, if any, between these two systems, but the results thus far are disappointing. Newborn rats were then studied, and such striking differences were noted between the lymphatic systems of the two ages that it was thought advisable to present as complete as possible an account of the entire system as evident at the time of birth.

The literature on the origin and the distribution of the lymphatic system is extensive; it will not be reviewed exhaustively here. Ranvier, in 1897, first injected mammalian embryos. Swaen and Brachet, in 1900, supported the idea of independent origin. Of the numerous American investigators, discussion has centered around Sabin, Lewis, Huntington, and McClure. Sabin ('02, '05, '08) has held that the entire systemic lymphatics arise first as a budding process from the embryonic veins of the cervical and inguinal regions. From the four sacs thus formed, the lymph channels of the entire body develop by continuous centrifugal growth to distal portions of the body. Lewis, in 1905, working on the rabbit, concluded that the origin is by several outgrowths from the venous system instead of four. Huntington and McClure, in 1907 and 1908, and Huntington, in 1911, affirmed the independent origin of the lymph channels from perivenous mesodermal extra-intimal spaces. These anlage unite to form complete channels which establish secondary connections with the venous system. This conforms with the theory of the independent origin of lymphatic endothelium and pronounces its ontogenesis as independent of hemal vasculogenesis.

The injection method was used entirely, and only macroscopic observations with the help of a 10 × binocular were employed. Relatively complete injections of the lymphatics of a newborn rat may readily be made through the skin, and it was found that the injection of 0.25 cc. of undiluted India ink into either of the three lymphatic channels at the base of the tail would completely fill the cutaneous and systemic channels of the posterior half of the body. The valves, at this time, are apparently non-functional; thus the injected material is easily forced through 'the afferent channels to the tip of the tail as well as to the distal parts of the legs. Likewise, a similar injection into the integument at the angles of the mouth or into the region just back of the ear will usually fill all of the cutaneous lymphatics and the systemic channels of the rest of the body. Only the finest cannulas, with an aperture measuring from 80 to 100 μ, were found satisfactory for these injections, the commercial needles being too large for insertion into the lymph channels.

RESULTS

For convenience of consideration the body of the rat is divided into four regions: cephalocervical, thoracic, abdominal, and caudal. The distribution of the superficial and deeper lymphatics will be considered under these general headings. The lymph nodes with their respective afferent and efferent channels will be considered as a unit within each section.

The cephalocervical region

If an injection is made slowly into the integument at each angle of the mouth and just back of the ear, the lymphatics of the head and neck are readily filled. The cutaneous lymphatics are filled, and the delicate small network of capillaries over the snout, around the eye, and the external ear as well as the face stand out distinctly (fig. 1 A). (In all figures the veins are indicated by a broken line and the lymphatic vessels by a solid line.) Microscopic examination of the integument shows that the lymph capillaries vary greatly in size at different levels; those of the deeper corium measure 50 to 100 μ in diameter, while those of the upper corium and epidermis have the smallest caliber. The median line of the head down to a transverse line connecting the eyes is devoid of cutaneous lymphatics.

From the integument, six or seven lymph channels in more or less definite positions pass to the musculature, and there connect with the systemic vessels (fig. 2 A). The lymphatics of the snout, the upper lip, and the cheeks unite to form a capillary plexus at the angles of the mouth, whence a series of two or three channels pass backward over the lower jaw, and onto the ventral surface of the geniohyoid muscle to the superficial cervical lymph node (fig. 2 A). Likewise, the lymphatics of the lower jaw, the lower lip, and the anterior floor of the mouth unite into one or more channels which pass back along the median ventral branch of the anterior facial vein to their respective superficial cervical nodes. In contrast to the adult condition, in which as many as six lymph nodes may be identified in the superficial cervical group, but a single pair exist in the newborn rat. These are slender ovate nodes, 3 mm. long and approximately 1 mm. wide, and lie obliquely on the geniohyoid and masseter muscles at the bifurcation of the external jugular vein into the anterior and posterior facials (fig. 2 A). Besides these channels, each superficial cervical node receives the lateral cervical cutaneous channels and the efferent channels from the parotid group of lymph nodes. This group lies either ventral or posterior to the ear, and consists of one or more smaller nodes (fig. 2 B). Likewise, the efferent ducts from the integument of the shoulder and the upper arm enter the corresponding superficial cervical node. Thus all of the lymph capillaries of the superficial parts of the head—the integument, mouth, sense organs, and musculature—unite to form larger channels, all of which lead to one of the two superficial cervical nodes. Posterior

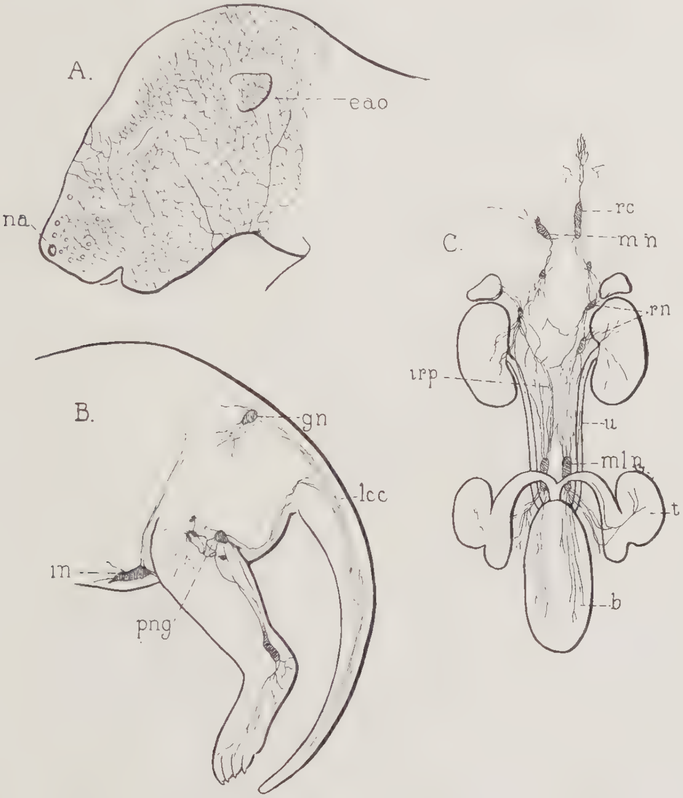

Fig. 1 A. Side view of head of newborn rat, showing cutaneous lymphatics. × 4. *eao,* external auditory organ; *na,* nasal aperture. B. Side view of the caudal region, showing portions of superficial and deeper lymphatics of hind leg. × 4. *gn,* gluteal node; *in,* inguinal node; *lcc,* lateral caudal channel; *png,* popliteal node group. C. Sketch to show lymphatic relations of the kidneys, ureters, bladder, and testes. *b,* bladder; *irp,* interrenal plexus; *mln,* median lumbar node; *mn,* mesenteric node; *rc,* receptaculum chyli; *rn,* renal node; *t,* testes; *u,* ureter.

to each of these, an intimate lymphatic plexus envelops the external jugular vein, but no vascular communications here were identified.

From this superficial part of the lymphatic system a series of small lymph capillaries pass laterally from the plexus around the external jugular vein, as well as from the caudal part of each cervical node, over the ventral surface of the cleidomastoid muscle to connect with the deeper cervical lymphatic channels (fig. 2 A). Just as the superficial cervical lymph capillaries are largely a unit with respect to the external jugular vein and its branches, so are the deeper cervical lymphatics largely a unit with respect to the internal jugular vein and its derivatives.

A varying number of nodes comprise this deeper cervical group. Lying just lateral to the internal jugular vein and the trachea, and dorsal to the sternomastoid and cleidomastoid muscles, is the largest node of the group (fig. 4 B). Associated with this are two or three smaller nodes, all connected by definite channels. The more anterior node of the group lies just medial to the petrosal cartilage, and receives the afferents from the deeper musculature of the jaw and the deeper portions of the mouth, as well as from the region of the inner ear. The two or three more lateral lymph nodes of this cervical group receive the afferent capillaries from the anterior trapezius and splenius muscles and from the deeper levator scapulae and others. Thus the deeper lymphatics of the neck all drain into the largest node of this group, whence a single efferent duct leads backward just lateral to the trachea to expand into an enlarged sac, which may be called the jugular lymph sac. This lymph sac, likewise, receives the efferent channels of the superficial system which pass upward over the lateral surface of the cleidomastoid muscle in the manner described. Thus the efferent channels of both the superficial and deeper cervical lymphatics come together at this somewhat enlarged jugular sac which, by a very narrow tap, is found to establish the only cephalic venous communication at the jugular bifurcation (fig. 4 A).

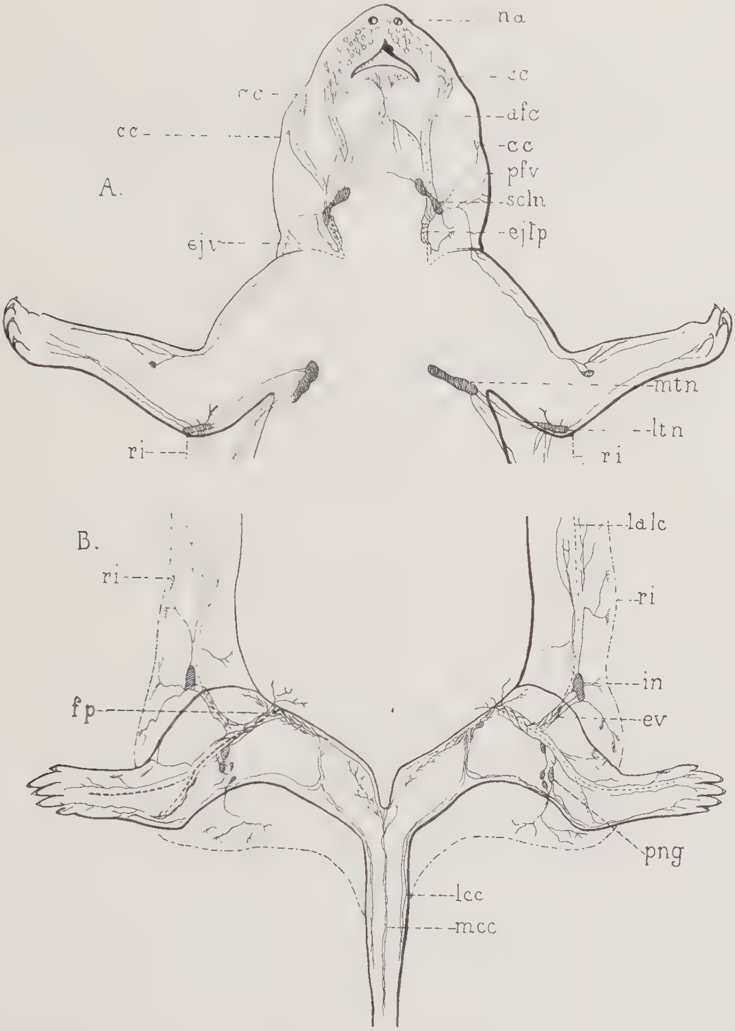

Fig. 2 A. Ventral view of cervical and thoracic regions, showing superficial lymph channels and nodes. × 4. *afc,* anterior facial lymph channel; *cc,* cutaneous lymph connections; *ejlp,* external jugular lymph plexus; *ejv,* external jugular vein; *ltn,* lateral thoracic node; *mtn,* median thoracic node; *na,* nasal aperture; *pfv,* posterior facial vein; *ri,* reflected integument; *scln,* superficial cervical lymph node. B. Ventral view of abdominal and caudal regions, with integument reflected, showing general distribution of the superficial lymphatics. × 4. *ev,* epigastric vein; *fp,* femoral lymph plexus; *in,* inguinal node; *lalc,* lateral abdominal lymph channel; *lcc,* lateral caudal channel; *mcc,* median caudal channel; *png,* popliteal node group; *ri,* reflected integument.

The thoracic region

The cutaneous lymphatics of the thorax are as complete as those of the head and neck and may be easily injected. Two pairs of lymph nodes, which are readily recognized in the superficial thoracic region, receive all of the lymphatic capillaries from the musculature of the superficial thorax. Of these nodes, the more external is the small one, measuring slightly less than 1 by 2 mm. It lies on the posterior surface of the triceps muscle, embedded in the surrounding subcutaneous adipose tissue, and receives the afferent ducts from the lateral thoracic and anterior abdominal integument, from the latissimus dorsi, the biceps and brachialis muscles, and the lymphatics of the forearm (fig. 2 B).

The more medial, somewhat deeper node is slightly more elongated and lies oblique to the primary body axis, beneath the pectoralis major muscle. It is approximately 4 by 1 mm. (fig. 2 A), and lies ventral to the long thoracic vein at its juncture with the ventral thoracic vein. The afferents to this node comprise the ventral abdominal cutaneous and the lateral abdominal cutaneous lymphatics, with a pair of lymphatic channels which parallel the long thoracic vein on the inner surface of the latissimus dorsi muscle (figs. 2 A, 3 B, and 4 A). Besides, the efferents of the lateral thoracic, or elbow node, enter this medial node, passing either along the axillary vein or directly along the triceps muscle across the anterior end of the latissimus dorsi (fig. 4 A). From this medial thoracic node in the adult, often termed the axillary, an exceedingly small efferent channel passes medially forward, ventral to the base of the subclavian vein, to join the jugular vein near the juncture of the two. In many cases this venous communication seemed to be incomplete and in others there apparently was no communication at all. It would appear that this tap may not be completely reestablished at this age, although conclusive evidence for the statement is lacking.

The deeper thoracic lymphatics are demonstrated with considerable difficulty. Within the thoracic cavity and lying ventral to the large vascular channels anterior to the heart, a group of very small thoracic lymph nodes are usually found (fig. 4 A). Each node is spherical and measures approximately 1 mm. in diameter. The posterior nodes of the group receive the afferent channels from the lungs and pleural

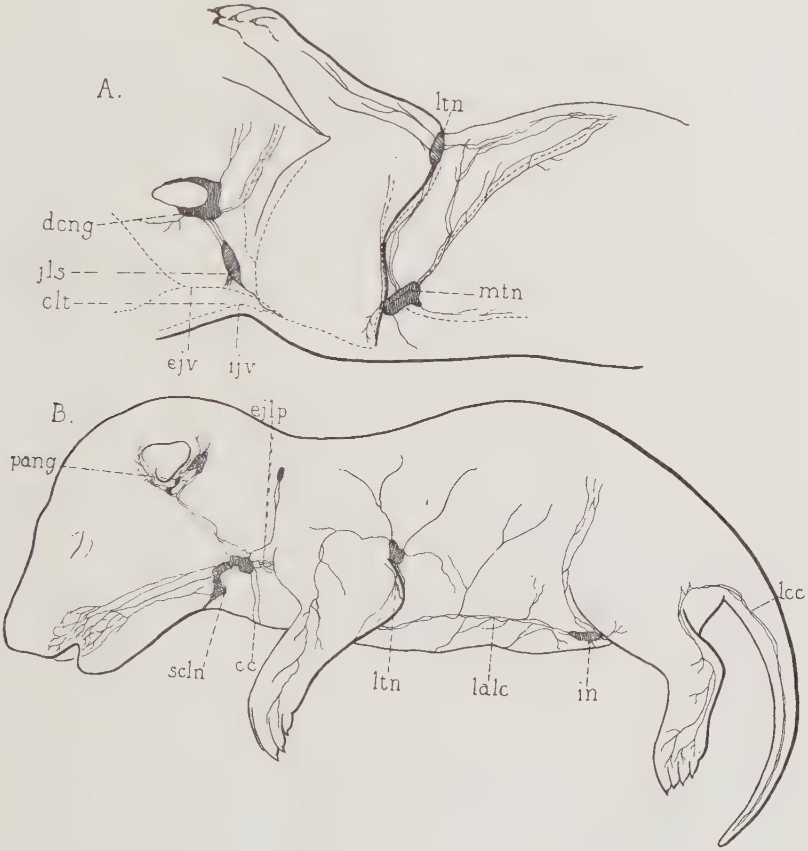

Fig. 3 A. Side view of cervical and thoracic regions, with fore leg elevated, showing deeper cervical channels and nodes. × 4. *clt,* cervical lymphatic tap; *dcng,* deep cervical node group; *ejv,* external jugular vein; *ijv,* internal jugular vein; *jls,* jugular lymph sac; *ltn,* lateral thoracic node; *mtn,* median thoracic node. B. Side view of entire animal with integument removed, showing general distribution of superficial lymphatics. ×4. *cc,* cutaneous lymph connections; *ejlp,* external jugular lymph plexus; *in,* inguinal node; *lalc,* lateral abdominal lymph channel; *lcc,* lateral caudal channel; *ltn,* lateral thoracic node; *pang,* parotid node group; *scln,* superficial cervical lymph node.

cavity, whereas the anterior nodes receive small lymph capillaries from the sternum. These are connected by small capillaries, and a single efferent channel from the more anterior node communicates with the venous system through one or more jugular taps near the cervical communication.

The abdominal region

The abdominal lymphatic channels of the integument and superficial muscular layers only are considered here. The lymphatics of the peritoneal cavity, and the deeper, more posterior capillaries of the caudal region with which they are connected, will be discussed in a subsequent paper.

The cutaneous lymphatics of the abdominal, the posterior thoracic, and lumbar regions may be readily injected through the larger lymph channels at the base of the tail. A single pair of ovate nodes, each of which measures approximately 2 by 1 mm., lie just below the integument, embedded in the adipose tissue of the inguinal region (figs. 2 B, 3 A, B, and 4 A). These are frequently lost in removing the integument, so closely is each node held, not only by the surrounding adipose tissue, but by the efferent cutaneous lymphatics which each receives. Efferent cutaneous channels from the pelvic region, the thigh, the sacral and lumbar regions, and those from the posterior parts of the abdomen empty into these inguinal nodes, from each of which there are two possible efferent channels for the lymph. The return circulation may pass forward, either along the prominent lateral abdominal lymphatic plexus to the axillary node of the thorax or through the channels which form a plexus around the epigastric vein, thence to the internal portion of the system with the femoral lymphatics of the hind leg (fig. 2 B).

The caudal region

The lymphatic capillaries of the caudal portion of the body are presented in a manner similar to that employed in the presentation of those of the cephalocervical region. A super-

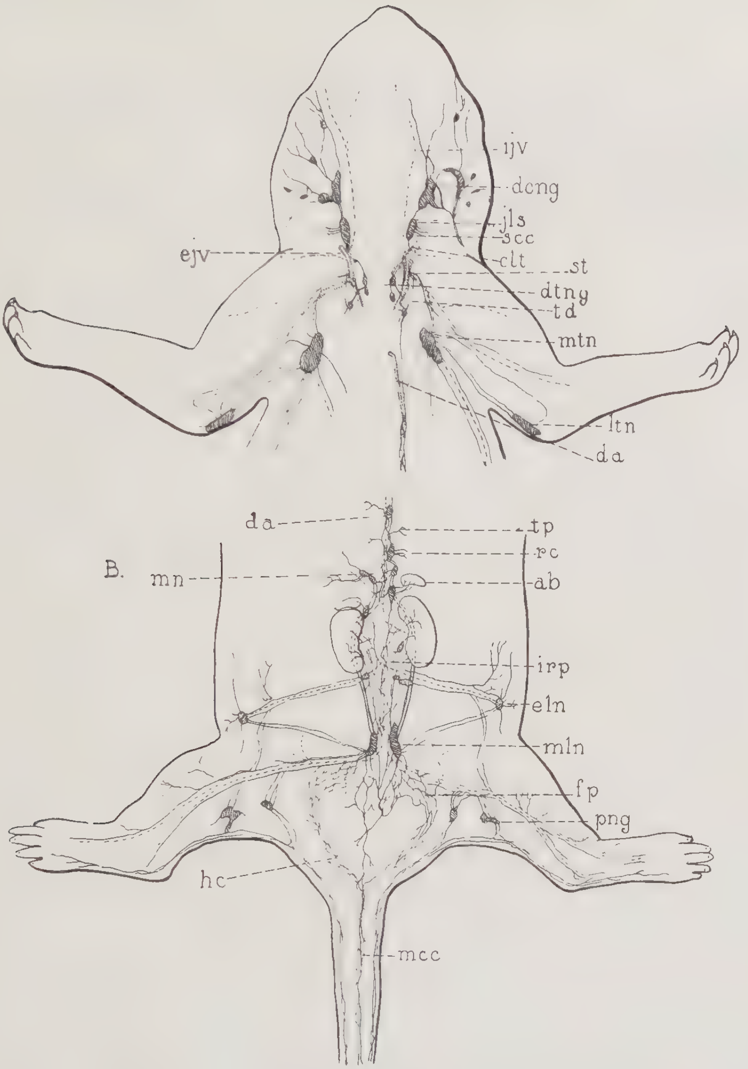

Fig. 4 A. Ventral view of cervical and thoracic regions, showing deeper lymph channels and related nodes. × 4. *clt,* cervical lymphatic tap; *da,* dorsal aorta; *dcng,* deep cervical node group; *dtng,* deep thoracic node group; *ejv,* external jugular vein; *ijv,* internal jugular vein; *jls,* jugular lymph sac; *ltn,* lateral thoracic node; *mtn,* median thoracic node; *scc,* superficial cervical channel; *st,* subclavian tap; *td,* thoracic duct. B, Ventral view of abdominal and caudal regions, showing deeper lymphatic channels and related nodes of the body musculature and peritoneal cavity. × 4. *ab,* adrenal body; *da,* dorsal aorta; *eln,* external lumbar node; *fp,* femoral lymph plexus; *hc,* hemorrhoidal channel; *irp,* interrenal plexus; *mcc,* median caudal channel; *mln,* median lumbar node; *mn,* mesenteric node; *png,* popliteal node group; *rc,* receptaculum chyli; *tp,* thoracic plexus.

ficial set of capillaries join the deeper ones, which in turn continue into the abdominal cavity, there to unite with the lymphatics of the peritoneal cavity.

The lymph capillaries of the tail (integument and musculature) unite to form three main groups of channels, one of which lies on the ventral surface of the musculature, and at the base of the tail divides into three sets of lymph capillaries, all entering the sacral region (figs. 2 B and 4 B). The other two groups of lymphatics lie one on each lateral surface of the tail. At the base of the tail two sets of lymph capillaries arise from each lateral group; the more medial of these continues forward over the thigh, then dips below the biceps femoris muscle to a small gluteal node embedded there (fig. 1 B). Continuing ventrally along the external surface of the thigh, the remaining capillaries coming from the tail join the popliteal node group in the posterior angle of the knee. The lymphatic capillaries of the hind leg are drained by two main groups of channels, the more prominent being the one which accompanies the femoral vein and its branches. Into it distally the efferent capillaries of the digits, the soleum, the anterior tibialis, and peroneus muscles are received, whereas more proximally the efferent capillaries from the popliteal node group, the medial thigh musculature, and the inguinal node enter (fig. 2 B). Along the caudal surface of the hind leg, and somewhat paralleling the posterior tibial vein, is a second lymphatic plexus. From this rete certain connections are made directly with the femoral lymphatic plexus, other connections occur through the popliteal node group, and still others continue medially to join the capillaries from the tail. In the latter case an extensive lymphatic plexus is formed over the musculature at the juncture of the body and the thigh. This plexus joins that around the femoral vein, so that all of the lymph capillaries of the hind leg, those of the lateral parts of the tail, and those of the posterior part of the abdominal integument pass into larger channels which enter the abdominal cavity through the femoral opening (fig. 1 B).

At the base of the tail, as suggested, three groups of lymph capillaries arise from the median ventral lymphatic plexus which drains a portion of this appendage (fig. 4 B). Of these, a median channel courses forward along the median dorsal surface of the rectum, receiving the hemorrhoidal lymph capillaries, and certain others from the urethra and neck of the bladder, while each lateral capillary group of the ventral caudal region courses forward through the pelvis, dorsal to the ischial cartilages, to join the femoral lymphatic plexus from the hind leg (fig. 4 B). A considerable lymphatic plexus is formed by the juncture of this enlarged femoral rete with that of the median hemorrhoidal channel, and it receives efferent capillaries from the base of the rectum, the urethra, portions of the bladder, and from the bases of the ureters. Likewise, lymph capillaries from the testes pass to this plexus (fig. 1 C).

Following the blood-vascular channels, and forming a plexus around them, all the lymphatic vessels of the tail and hind legs converge to the median lumbar lymph node of their respective side (fig. 4 B). These lumbar nodes, which lie just anterior to the bifurcation of the dorsal aorta into the arteries of the legs, are larger than many of the other nodes, and receive, besides all the efferent vessels of the caudal region, those from a smaller pair of external lumbar nodes which lie distally near or on the lumbar vein. Each spherical external lumbar node, not always present on each side, receives the lymph capillaries from the hypaxial musculature of the region, and, also, certain of the efferent channels from the gluteal lymph node which pass ventrally through the thigh musculature. Thus, perhaps, a small portion of the returning lymph of the tail and of the thigh and sacral regions may pass through the gluteal to the external lumbar node and thence to the renal group of lymphatics, whereas the major portion will pass through the median lumbar nodes in the manner described.

The efferent channels of these median lumbar nodes continue cephalad, ventral, and lateral to the dorsal aorta, forming a complex plexus around it. Thence they pass into the interrenal mesentery, where, ventral to the renal veins and arteries, they continue into an extensive plexus of lymph capillaries (fig. 4 B). Diligent search was made for venous communications here, like those in the adult common rat, in which lymphatic connections with the renal vein were observed; no communications were found, although this does not preclude their existence. A group of four or five small renal nodes occur in the region between the kidneys and adjacent to the adrenal bodies. These receive the efferent channels from the renal capsules and the adrenal bodies, also certain afferent channels from the external lumbar node (fig. 4 B).

Suspended in the dorsal mesentery, slightly ventral and anterior to the renal group, is the large mesenteric node, connected by several lymph capillaries to the interrenal lymph plexus and to the renal nodes. No attempt has been made in this study to obtain conclusive evidence concerning the extent of the lymphatic development of the alimentary tract. Material injected into the lymphatic system filled capillaries which could be traced from the mesenteric node along the mesenteric vein and other venous channels toward the ileum, stomach, and liver. That these channels are incomplete at birth does not necessarily follow. On the other hand, a study of a rat two days old, when the alimentary tract was functioning, showed clearly the lymph channels of the ileum with the ileocecal lymph node and smaller mesenteric nodes. These latter structures were not identified in the rat at birth.

Slightly anterior to the renal group and to the mesenteric node, and lying to the left of the dorsal aorta, is an enlarged sac, the receptaculum chyli. Efferent channels of the interrenal lymph plexus, the renal nodes, the mesenteric node, and certain nodes from the dorsal posterior thoracic musculature, lead to the receptaculum. Continuing forward from this are a large number of small capillaries which form a plexus around the dorsal aorta and the adjacent tissue, and which will be called the thoracic plexus. A thoracic duct, as such, could not be said to exist here, although more anteriorly there is a definite channel. Many small efferent lymph capillaries from the intercostal regions and from the walls of the azygos veins join the thoracic plexus, which, besides covering the parts of the aorta, course around the intercostal arteries.

Anteriorly the thoracic duct is formed by the fusion of these many capillaries of the thoracic plexus. It follows the aorta, and at the arch courses slightly to the left, passes dorsal to the subclavian vein of that side and along the lateral surface of the esophagus to enter the venous system near the jugular bifurcation (fig. 4 A).

SUMMARY

The lymphatic system in the newborn rat is relatively complete, as was determined in this study by injecting the lymphatic channels with an opaque medium. The valves do not function at birth, which made it readily possible to fill the systemic lymphatic channels by subcutaneous injections. These injections were most satisfactorily made through the integument at the angles of the mouth, back of the ear, and at the base of the tail.

Lymph vessels are apparently more numerous in the newborn rat and less inclined to follow the course of the venous channels than in the adult. On the other hand, lymph nodes appear to be less numerous than in the adult. In the superficial cervical group several nodes may be identified in the adult, whereas only a single node can be found in the newborn rat.

A jugular lymph sac was found to persist in many rats studied. This connects by the jugular tap with the venous system. All the efferent channels of the superficial and deeper cervical groups lead to the jugular sac.

Lymphatics of a part of the tail, hind legs, penis, testes, and bladder, all converge to the medial lumbar nodes.

There is a complex plexus of lymphatic capillaries in the interrenal region, lying ventral to the blood vascular channels and extending as far forward as the adrenal bodies. No venous communications were identified here.

Lymph capillaries from the stomach, spleen, and pancreas were not identified in the newborn rat, but this does not preclude their existence.

The thoracic duct is more largely a plexus in the posterior thoracic region, but as a definite channel it passes dorsal to the subclavian vein and lymph approach of that side to its venous communication with the left common jugular vein.

December 31, 1924.

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THE DEVELOPMENT OF THE DIGESTIVE TRACT AND ITS ARTERIES IN REPTILES

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TWENTY-TWO FIGURES (THREE PLATES)

In marked contrast to their rather uniform arrangement in mammals, the arteries supplying the digestive tract of reptiles pursue very different courses in the various representatives of that order. The subject attracted the attention of the pioneer comparative anatomist and embryologist, Rathke, and later of that diligent investigator of the vascular system, Hochstetter ('98). Together these two noted workers examined and described ninety-three species. A glance at plate 1, composed of figures which (with the exception of fig. 5) are copied from Hochstetter's paper, will convey an idea better than any description of the complexity of this part of reptilian anatomy.

That these most diverse arterial patterns began in a common embryonic stage and were produced by the accentuation of one or more developmental tendencies are suppositions that naturally occur to any student of comparative embryology. Rathke recognized, in a general way, that the various arterial systems which he found were modifications of a common plan. The notion was more clearly expressed and supported with embryological evidence by Hochstetter ('98, '06). Hochstetter described the early position and later wandering of the coeliac artery in Anguis fragilis, and pointed out that the varying relations of the coeliac to the mesenteric artery in other reptiles could be explained by assuming a similar process in their cases.

In the course of an extended study of the embryology of the common fresh-water turtles, Chrysemys marginata and C. picta, I have worked out the development of the digestive tract and the arteries that supply it. The early stages of the turtle show rather clearly a group of embryonic vessels from which the adult arterial systems of the digestive tracts of all other reptiles may readily have been derived, and, moreover, exemplify the developmental tendencies by the operation of which the adult arterial arrangements of the various forms can be produced. A brief report of the findings in the turtle, and their application to reptiles in general, should therefore be of interest to comparative embryologists.

DEVELOPMENT OF THE INTESTINE OF TURTLES

To follow the history of the arteries to the digestive tract, the development of the intestine must first be described. The primitive gut-tube is formed, as in other vertebrates, by a gradual encroachment of the foregut and hindgut upon the entodermal sac (fig. 10). As the yolk sac and stalk are differentiated, they retreat ventrally, and come to lie outside the abdominal cavity. The yolk stalk is always short, and the middle segment of the gut is drawn out into a U, the limbs of which lie in the midsagittal plane (fig. 11). The primary intestinal loop is thus formed. At the same time a dilatation appears in the foregut, to mark off the future stomach. That organ lengthens rapidly, and twists to the right, thereby throwing its pyloric end and the first part of the intestine into a spiral turn, which is similar to that found in birds, and like it can be called the duodenal loop (fig. 12 at *D*). The portion of the intestine caudal to the primary loop is unaffected by the changes above it. In a 9.6-mm. embryo (fig. 12) this otherwise unaltered segment shows a general increase in diameter which marks it off as the future large intestine.

Then follows a long period which is chiefly occupied with the further development of the primary loop. In the 9.6-mm. embryo already referred to, the proximal or outgoing limb of the loop lies cranial to the distal or incoming limb. The entire loop now undergoes a dextral rotation about the yolk stalk as an axis, which is the same sort of rotation as occurs more familiarly in mammals. The rotation is well begun at 10.6 mm. (fig. 13) and is complete at 27 mm. (fig. 14). The outgoing limb then lies caudal to the incoming one—just the reverse of the earlier relation. In the latter part of the period of rotation, the first intestinal coils appear. Two sets of coils are found at 27 mm., placed at the beginning and end of the primary loop, respectively.

The position of the two sets of intestinal coils enables one to locate the part formed by the loop in the intestine of the adult. In the turtle, as in the chick (Duval, '89), the loop itself is wholly converted into small intestine. If the turtle and chick are representative forms, Sauropsida differ considerably from mammals in this respect. In man (Mall, '97) and in the pig (Lewis, '03, and Lineback, '16) the greater part of the incoming limb of the primary loop is added to the large intestine, and the caecum is placed not far from the yolk stalk.

In the turtle the large intestine is entirely caudal to the primary loop. A rudimentary caecum appears at 27 mm. (fig. 14), and is more marked then than in the adult. The spleen is definitely present in a 10.6-mm. embryo (fig. 13), and is found in its usual relation to the stomach and dorsal mesentery. In the turtle it is also closely related to the caecal end of the large intestine.

In an embryo of 27 mm. and in one of 32 mm., the oldest available, the caecal end of the large intestine, the spleen, and the distal ileal coils of the small intestine lie in the upper left-hand corner of the abdominal cavity. In the adult Chrysemys picta (fig. 16) and Chelydra serpentina (fig. 15) all these parts lie in the upper right-hand corner, and are fastened to the duodenum and pancreas by mesenteric attachments. In what must constitute a third stage of development, the large intestine lengthens and makes its way across the duodenum. The spleen and distal ileal coils of the small intestine accompany the large intestine in this migration, and all become lodged in the position in which they are found in the adult. When development is complete, the turtle boasts of a transverse and a descending colon—unique features for a non-mammalian vertebrate.

There is an interesting correspondence in the development of the large intestine in the turtle and man. In the human embryo, as already noted, the large intestine forms much of the incoming limb of the primary loop. When the loop rotates, the transverse colon is at once brought into position. The ascending colon is then formed by a downward growth of the large intestine, pushing the caecum into the lower right-hand corner of the abdominal cavity. In short, much the same developmental process occurs in both man and the turtle, though there is a fundamental difference in the make-up of the primary intestinal loop.

DEVELOPMENT OF THE MESENTERIC ARTERY

In the beginning the entoderm is supplied by the usual series of paired ventrolateral branches of the dorsal aorta. With the establishment of the foregut and hindgut, the numerous paired vitelline vessels give way to a row of single ones. Such is the arrangement in a 5.9-mm. embryo (fig. 10). With the development of the primary intestinal loop, the blood supply is again altered. At 9.5 mm. (fig. 11) a single mesenteric artery has emerged from the row of vitelline vessels. Transitional forms of the artery, with two or more roots, can be found (e.g., as in fig. 17).

The mesenteric artery of the 9.5-mm. embryo (fig. 11) has, in addition to its usual yolk-sac branches, two others of much future importance. Near its origin it gives rise to a tiny coeliac artery, and near its end, to another vessel, which is to become the adult colic artery. The coeliac artery has but a temporary connection with its parent trunk; it will be dealt with below. The colic artery always remains a branch of the mesenteric—in the turtle, at least—and reaches its adult position after a very peculiar development.

The future colic artery first appears as a branch of the mesenteric, which leaves the parent vessel just proximal to the yolk-sac vessels, and which courses caudally in the mesentery of the distal segment of the intestine (fig. 11, *R.r.*). A comparison of figure 11 with figure 10 shows its genesis. It is formed from a longitudinal anastomosis of the vitelline arteries, already indicated in the 5.9-mm. embryo (fig. 10). When the vitelline arteries disappear, the anastomosis remains, takes secondary origin from the mesenteric trunk, and becomes the branch in question. With the increase in size of the primary intestinal loop, the mesenteric artery is necessarily lengthened, but its increase in length occurs in the part proximal to the origin of the colic branch. The origin of the branch is therefore carried ventrally outward. The branch itself is greatly lengthened in this process and is converted into a long recurrent ramus. As such it is found in a 9.6-mm. embryo (fig. 12, *R.r.*).

At 10.6 mm. (fig. 13) the primary loop begins its dextral rotation. The yolk-sac branches of the mesenteric artery rotate also. The stem of the artery is twisted, and the origin of the now recurrent ramus is carried from the under to the upper side of the mesenteric artery. By this time the spleen is definitely developed. A splenic artery appears at the same time. It is a branch of the recurrent ramus.

The extreme reentrant angle, at which the recurrent ramus takes origin in the 10.6-mm. embryo, is made less acute at 27 mm. (fig. 14) by a migration of the origin of the branch up the parent stem. Meanwhile the coils of the small intestine have been formed. Those constituting the proximal or duodenal set are supplied by new twigs from the mesenteric trunk. The ileal coils are supplied by branches of the recurrent ramus.

Between the 27-mm. stage and the adult comes the period of rapid elongation of the large intestine and the migration of its caecal end, together with the spleen and ileal intestinal coils, across the duodenum into the upper right corner of the abdominal cavity, as already described. These form the area of distribution of the recurrent ramus. Its branches migrate with the intestine and spleen, and the recurrent ramus acts as a radius vector for the movement. When the migration is completed, the reentrant angle at the origin of the recurrent ramus is all but wiped out, and the vessel, now the colic artery of Bojanus, can be found as a slightly recurrent branch of the mesenteric artery in Chelydra (fig. 15), and as a similarly placed vessel, slightly obscured by the encroaching of its branches upon it, in Chrysemys picta (fig. 16).

So far as known, there is no branch of the embryonic mesenteric artery in mammals that corresponds to the colic of the turtle. Instances of it are probably widely scattered, however, through reptiles. The mesenteric artery of a 10-mm. embryo of Iguana tuberculata has a branch which takes a course much like that of the recurrent branch of the 9.6-mm. turtle; a very definite colic branch can be found also in embryos of Sphenodon punctatum of 7.9 mm. and 17.4 mm.

As Hochstetter ('06) found in Anguis, the coeliac artery begins also as a branch of the mesenteric artery. It can be found in the turtle of 6 mm. (fig. 17) as a very fine branch which runs directly craniad as far as the dorsal pancreas. At this point it divides into a ventrally directed coeliac artery proper, which runs along the right side of the dorsal pancreas, and a gastro-epiploic, which continues the course of the parent stem.

The common trunk from the mesenteric artery for the coeliac and gastro-epiploic arteries is a short-lived vessel. Its two branches speedily acquire independent origins from the dorsal aorta (fig. 19). The way in which the change takes place is obscure. Whether the common trunk migrates to the aorta, as Hochstetter states, and is absorbed by its branches, or whether a new common root from the aorta replaces the old one, to be in its turn absorbed, as the 9.5-mm. embryo (fig. 18) would suggest, could not be decided with the material available.

The relation of the three now independent arteries—mesenteric, coeliac, and gastro-epiploic—to the aorta is complex.

In younger embryos, e.g., 9.6 mm. (fig. 19), all three leave the dorsal aorta far below the union of the dorsal aortae. The point of union of the aortae and the origins of the three arteries gradually approach each other, so that in a 19-mm. embryo the arteries arise from the left dorsal aorta. They do the same in the 27-mm. embryo (fig. 21) and in the adult (fig. 22). Such an arrangement is the product of two movements. The origins of the arteries, which seem to move upward, really move downward. The origin of the mesenteric in a 9.5-mm. embryo lies between the ninth and tenth spinal ganglia. At 10.6 mm. it lies between the tenth and eleventh, and at 27 mm., opposite the sixteenth ganglion. At the same time there is a real secondary splitting of the dorsal aorta into right and left portions which are continuous, respectively, with the ununited dorsal aortae. The splitting proceeds downward faster than the downward migration of the arterial roots, overtakes them, and transfers them to the left aorta.

The terminal ramifications of the coeliac and gastro-epiploic arteries develop in intimate relation to each other. Each connects with a vascular plexus which surrounds the stomach and becomes especially developed on its right side and along its greater and lesser curvatures. The gastro-epiploic artery ends in the plexus along the greater curvature (fig. 19); the coeliac connects with the plexus near the pylorus through a special tap branch (x in fig. 19).

Out of the gastric plexus two longitudinal anastomotic channels differentiate along the two curvatures of the stomach, respectively. That along the greater curvature becomes the continuation of the gastro-epiploic artery (fig. 20); that along the lesser becomes its branch, the gastric artery. The gastric artery retains some early hepatic branches and supplies the liver as well as the stomach. Both gastric and gastro-epiploic vessels communicate below, through the tap branch (x), with the coeliac artery. This tap branch increases in size and importance, and in the adult becomes the terminal portion of the coeliac artery. The part which was terminal in the embryo is then the hepatoduodenal artery. The growth and branching can be followed in figures 19, 20, 21, and 22. It divides into duodenal and hepatocystic arteries, the latter of which in turn divides into hepatic and cystic vessels. Pancreatic arteries are numerous; the chief one appears at 10.6 mm. (fig. 20) as a branch of the tap branch of the coeliac.

In the 27-mm. stage (fig. 21) one can identify a small cardiac branch of the left aorta, which passes to the cardiac end of the stomach. In the turtle it is a variable artery and of little importance. In other reptiles it is a much larger vessel and may be multiple.

SUMMARY

The fairly complicated arterial system of the digestive tract in the turtle is derived from a single mesenteric artery, which arises from the dorsal aorta. This mesenteric artery, in addition to its usual branches to the yolk sac, has a colic branch which runs caudally behind the large intestine and a coeliac branch which passes cranially to supply the stomach and adjacent glands. The latter branch divides into coeliac and gastro-epiploic arteries, and with the aid of a gastric plexus elaborates the blood supply to the stomach, liver, and pancreas. The transformation of the mesenteric artery takes place in stages, so that not all these elements can be found well developed in one embryo—at least not among those at my disposal. Figure 5, a combination of figures 11, 17, 18, and 19, gives a somewhat diagrammatic picture of the complete embryonic mesenteric artery.

In later growth three developmental tendencies guide the elaboration of the mesenteric system. They are as follows:

1. The branches of the embryonic mesenteric artery tend to split off and acquire separate origins from the aorta.
2. The whole system of vessels, whether divided or not, tends to migrate down the aorta.
3. The aorta tends to redivide into two.

DISCUSSION

If one keeps in mind the embryonic mesenteric system of Chrysemys and the developmental tendencies that control its later history, and then turns to the eight figures of Hochstetter in plate 1, he will see that the early stage of the turtle has a wide significance. The mesenteric artery and its chief branches, the coeliac and colic arteries, are the vascular units of the ideal central type that Rathke sought for. Whether they are combined in a single system in any other reptilian embryo is, of course, unknown; it is probable that the turtle shows an extreme condition of combination and reduction. At all events, by taking these arteries as derived directly or indirectly from the yolk-sac vessels, and given the developmental tendencies enumerated above, the vascular system of the digestive tract of any reptile can be evolved.

For Lacerta the adult mesenteric artery is essentially the embryonic vessel of the turtle.

The three major vessels, mesenteric, coeliac, and colic arteries, can develop in several adult combinations. If the mesenteric and colic arteries unite and the coeliac is an independent vessel, there is produced the arrangement found in Sphenodon and Anolis. If the coeliac and colic are united and the mesenteric is separate, the condition found in Varanus results. If all three vessels have separate origins, the result is as in the four remaining reptiles, Hydrosaurus, Anguis, Chamaeleon, and Iguana.

The independent coeliac and colic arteries do not bear a constant relation to the mesenteric in all reptiles. Such displacements of the vessel origins would come to pass in the general downward migration of the whole mesenteric system, if the rates of progress of its components were not equal.

In Varanus and Hydrosaurus the arteries arise not from the abdominal, but from the left dorsal, aorta. Such a variation can be explained as the result of the developmental tendencies well illustrated in Chrysemys. Simultaneously with the downward migration of its branches, the aorta secondarily divides. In these reptiles the subdivision proceeds so fast that the branches are overtaken and transferred to the left aortic arch.

The ramifications of the coeliac and gastro-epiploic arteries in each instance are combinations of the embryonic coeliac artery and the gastric plexus, such as occur in the turtle, with the varying assistance of the cardiac artery or arteries. In the turtle there are two pathways for blood flow to the liver, one through the gastric artery and the other the continuation of the coeliac artery. The first pathway is emphasized in Anolis and Anguis; the second in Sphenodon, Varanus, and Hydrosaurus. In Chamaeleon and Iguana the coeliac pathway is utilized, but it is apparently secondarily captured by the mesenteric artery proper.

Until special studies have been made, it is hardly useful to particularize further. But we can reasonably conclude from what has already been seen that the dissimilar arterial systems of the digestive tract in various reptiles are derived from a group of embryonic vessels very much the same in all forms, that and the adult pattern in each case is the result of like developmental tendencies working in varying degrees, both embryonic vessels and developmental tendencies being clearly exemplified in the early stages of the turtle.

In conclusion, I must express my obligation to the Department of Anatomy of the Harvard Medical School for the means and material for research and publication, and especially to Prof. F. T. Lewis, with whom I have studied for some years.

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ABBREVIATIONS

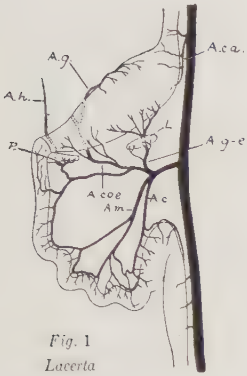
A., aorta
A.c., colic artery
A.ca., cardiac artery
A.coe., coeliac artery
A.cy., cystic artery
A.d., right dorsal aorta
A.du., duodenal artery
A.g., gastric artery
A.g-e., gastro-epiploic artery
A.h., hepatic artery
A.h-c., hepatocystic artery
A. h-d., hepatoduodenal artery
A.m., mesenteric artery
A.m.i., inferior mesenteric artery
A.pa., pancreatic artery
A.py., pyloric artery
A.s., left dorsal aorta
C.i-c., ileocolic junction
D., duodenum
D.h-e., hepato-enteric duct
D.v., yolk stalk
E.a., foregut
E.p., hindgut
G., stomach
I.cr., large intestine
I.t., small intestine
L., spleen
P., pancreas
P.d.. dorsal pancreas
P.v.d., right ventral pancreas
P.v.s., left ventral pancreas
Py., pylorus
R.h., hepatic ramus of gastric artery
R.r., recurrent ramus; the adult colic artery
S.v., yolk sac
V.f., gall bladder
x., tap branch of the coeliac artery

PLATE 1

EXPLANATION OF FIGURES

1 to 4 and 6 to 9 Semidiagrammatic figures showing the arteries to the digestive tract in various reptiles. Copied from Hochstetter ('98) and relabeled. 1, Lacerta viridis; 2, Sphenodon punctatum; 3, Anolis carolinensis; 4, Varanus niloticus; 6, Hydrosaurus salvator; 7, Anguis fragilis; 8, Chamaeleon gracilis; 9, Iguana tuberculata.

5 Diagrammatic reconstruction of the embryonic mesenteric artery of Chrysemys marginata, based on figures 11, 17, 18, and 19.


A.g.
A.h.
P.
A.ca.
L
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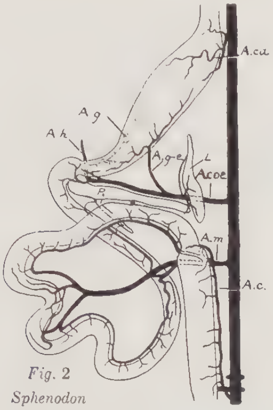
A.coe
A.m.
A.c

Fig. 1
Lacerta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A.c.

Fig. 2
Sphenodon

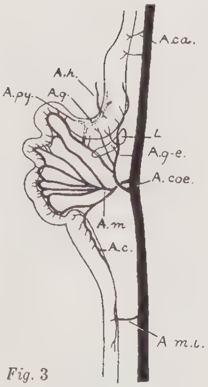

A.ca.
A.h.
A.py.
A.g.
L
A.g-e.
A.coe.
A.m
A.c.
A.m.i.

Fig. 3
Anolis

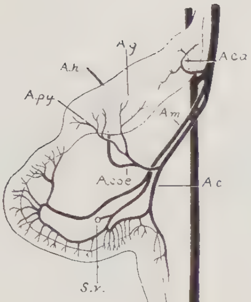
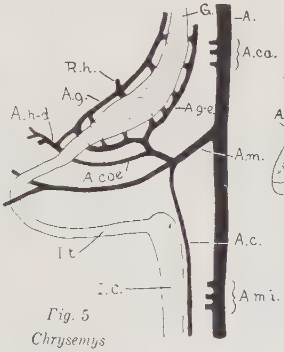

A.g
A.h
A.ca
A.py.
A.m
A.coe
A.c
S.v.

Fig. 4
Varanus


G.
A.
A.ca.
R.h.
A.g.
A.h-d
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I.c.
A.m.i.

Fig. 5
Chrysemys

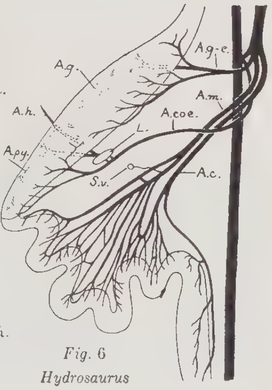
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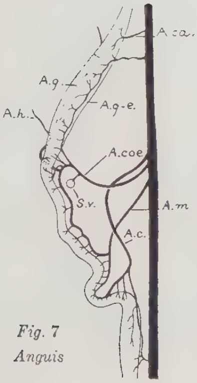
Fig. 6
Hydrosaurus


A.ca.
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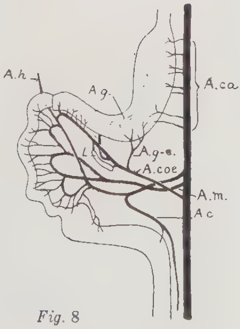
A.m
A.c.

Fig. 7
Anguis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A.g.
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L
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A.c

Fig. 8
Chamaeleon

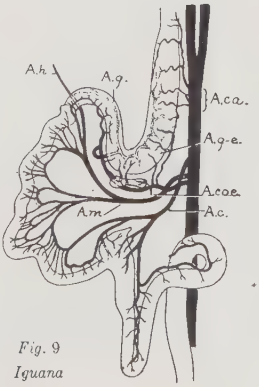

A.h
A.g.
A.ca.
A.g-e.
A.coe.
A.c.
A.m

Fig. 9
Iguana

PLATE 2

EXPLANATION OF FIGURES

10 to 14 Graphic reconstructions of the digestive tract and mesenteric artery in embryos of Chrysemys marginata. 10, 5.9 mm. Harvard Embryological Collection, series 1058; 11, 9.5 mm. H. E. C., series 1073; 12, 9.6 mm. H. E. C., series 1079; 13, 10.6 mm. H. E. C., series 1087; 14, 27.0 mm. H. E. C., series 1096.

15 Intestine and mesenteric artery of an adult Chelydra serpentina.

16 Intestine and mesenteric artery of an adult Chrysemys picta.

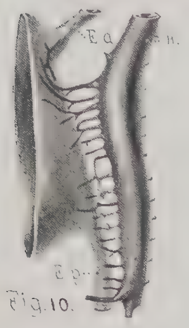


Fig. 10.

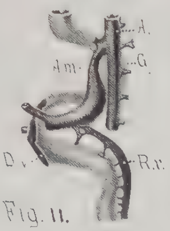


Fig. 11.

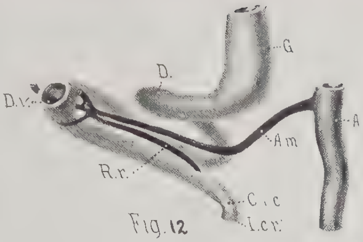
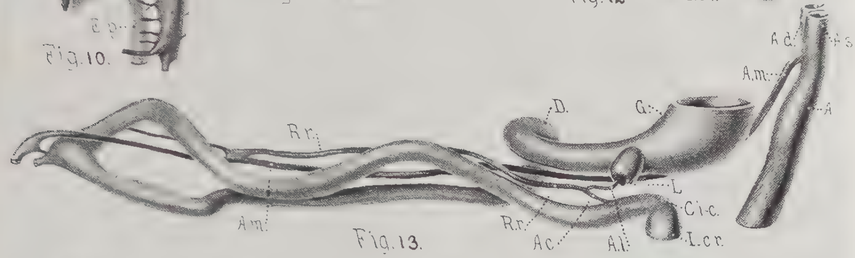

G
D.
D.v.
A
Am
R.r.
C.i.c
I.cr.

Fig. 12


A.d.
A.s
A.m.
A
D.
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Am.
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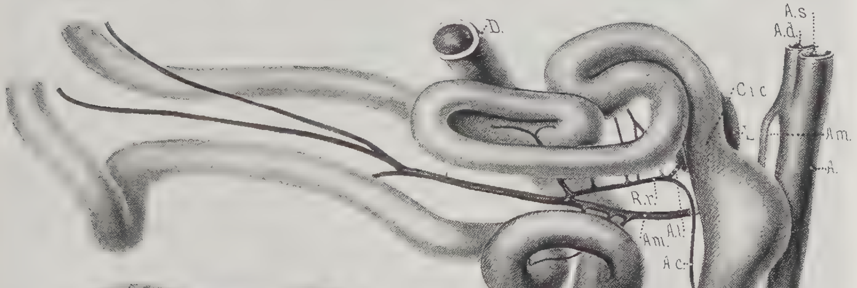
Ac
A.l.
I.cr.

Fig. 13.


D.
A.s
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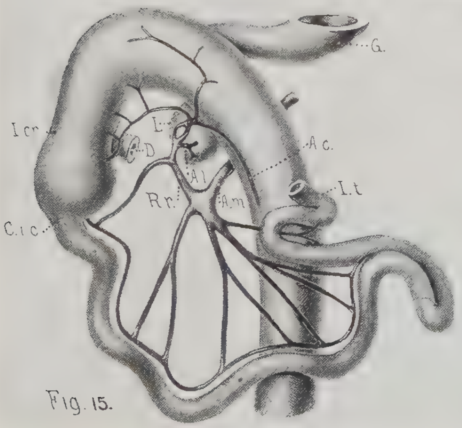
Ac
I.cr.

Fig. 14


G.
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L
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R.r.
Am
I.t
C.i.c

Fig. 15.

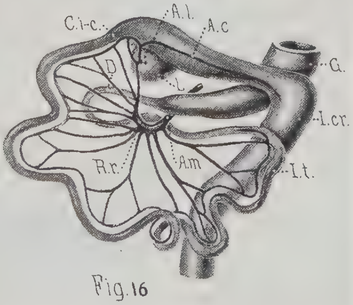

C.i-c.
A.l.
A.c
G.
D
L
I.cr.
R.r.
Am
I.t.

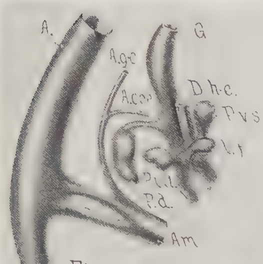
Fig. 16

PLATE 3

EXPLANATION OF FIGURES

17 to 21 Graphic reconstructions, supplemented by wax models, of the stomach and coeliac and gastro-epiploic arteries in embryos of Chrysemys marginata. 17, 6.0 mm. H. E. C., series 1067; 18, 9.5 mm. H. E. C., series 1073; 19, 9.6 mm. H. E. C., series 1079; 20, 10.6 mm. H. E. C., series 1087; 21, 27.0 mm. H. E. C., series 1096.

22 Stomach and coeliac and gastro-epiploic arteries of an adult Chrysemys picta.


A.
G
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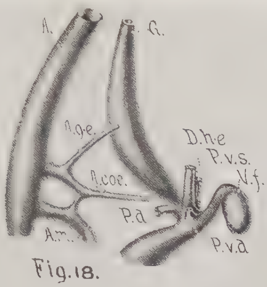
D.h.e.
P.v.s
N.f
P.v.d.
P.d.
Am

Fig. 17.


A.
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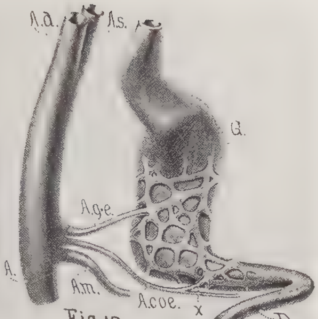
P.v.d

Fig. 18.


A.d.
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A.
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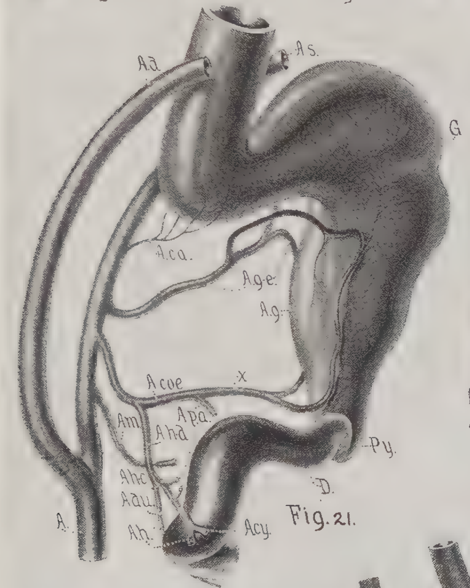
x
D.

Fig. 19.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x
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A.p.a.
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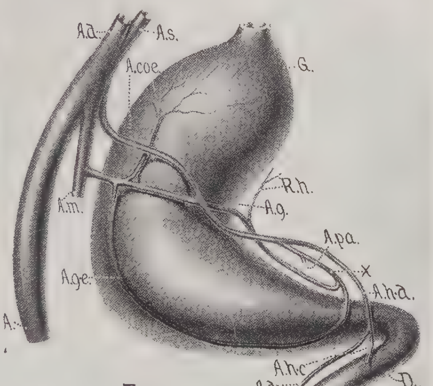
A.d.u.
A.
A.h.
A.cy.
Py.
D.

Fig. 21.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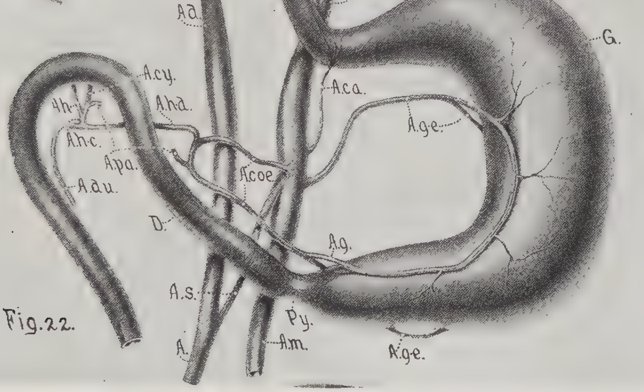
A.d.u.
D.

Fig. 20.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A.h.d.
A.h.
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A.ca.
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A.p.a.
A.coe
A.d.u.
D.
A.g.
A.s.
Py.
A.
A.m.
A.g.e.

Fig. 22.

THE OESTROUS CYCLE IN THE MARE AND SOME ASSOCIATED PHENOMENA

EDWIN SEABORN
London, Canada

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INTRODUCTION

A very favorable opportunity of studying the oestrous cycle in the mare, together with the gross and microscopic findings in the ovaries and uteri of these animals at definitely known periods throughout the cycle, was afforded the writer while in Paris, France, in the summer of 1923. Inasmuch as such material is difficult to obtain under ordinary circumstances, especially material from mares where the exact day in the oestrous cycle is known, it is felt that this study may be of value to workers in this field, particularly since interest in the phenomenon of oestrus in the mammals is now undergoing a marked reawakening.

The mares were kept in the stable under good conditions and were under observation for some time, so that their sexual behavior could be carefully noted and the exact time of the oestrous period could be accurately determined for each animal. Willingness to accept the male was taken as an indication of the oestrous period, and the animals were presented to the male daily, without allowing copulation to occur except in two cases—one killed immediately and the other on the second day following. The accurate determination of the exact stage of the cycle is of the utmost importance, for it enables us to correlate with the greatest exactness the external objective manifestations of the various stages with the gross and microscopic anatomical changes of the sexual and other organs taking place in these stages.

The oestrous cycle in the mare lasts twenty-four days. It may be divided into a period of rest, lasting eight days; a prooestrous period of three days, during which the animal exhibits external evidences of heat, but does not accept the male; an oestrous period of three days, characterized by decrease in amount of mucous discharge from the vulva and willingness to mate, and a metoestrous period of ten days.

Animals whose time of oestrus had been accurately determined were killed at various times throughout the cycle, so that material from all stages was available. The uteri and ovaries were removed, their gross appearance noted, and microscopic preparations made from them. In the gross examination of the uterus, it was first opened and the condition of the mucosa carefully noted and recorded. Particular attention was addressed to its looseness and flabbiness, color, vascularity, and oedematous condition. The ovaries of every animal were weighed separately, both untouched and after the follicles had been evacuated. The following report is based upon the findings in this accurately timed and well-preserved material. The Fallopian tubes, suprarenals, thyroids, hypophysis, and a portion of the vagina were also removed and examined.

In all, nineteen mares were so studied. While all of these could be utilized to show the progression of changes in the cycle, for the sake of brevity only six will be described in some detail, since these are all typical cases, illustrating all the important stages of the oestrous cycle. The stage of the cycle at which the mare was killed and the number of each specimen are given in the following table:

Fourteenth day following oestrus (rest),	mare no. 404
First day of prooestrus,	mare no. 398
First day of oestrus,	mare no. 459
Third day of oestrus,	mare no. 51
Second day following oestrus,	mare no. 468
Eighth day following oestrus (metoestrus),	mare no. 416

DESCRIPTION OF THE CASES

Mare 404—killed on the fourteenth day following oestrus

The opened uterus shows a mucosa of a faint bluish color, containing a minimum of moisture. The folds of the mucosa are not voluminous and do not hang down of their own weight when the uterus is held reversed. When the mucosa is cut, the tissue does not spread away from the cut edge and extend itself on the table—a process which we have called 'flowing.' The blood vessels of the cut mucosa are not visible with the naked eye.

Under the microscope, sections show the following characteristics: the superficial cells are cuboidal, with a small content of cytoplasm; the proportion of stroma is small and there is an almost total absence of lymphocytes.

The left ovary weighs 55 grams and contains four small Graafian follicle cysts, together with a corpus luteum very far advanced in degeneration. After evacuation of the cysts the ovary weighs 47 grams.

The right ovary weighs 38 grams and contains six small Graafian follicle cysts. After emptying the latter the ovary weighs 31 grams. The uterus and ovaries are considered to be in that state of low activity characterized as 'rest.'

Mare 398—killed on the first day of prooestrus

The uterine mucosa is of faint bluish color and is somewhat firm in texture. It is not pendulous and on section does not 'flow.' Under the microscope, we find great mitotic activity in the epithelium and beginning degeneration of the superficial stroma. Lymphocytes are frequently seen.

The left ovary weighs 33.50 grams and, after emptying the cysts, 29.50 grams. The right ovary weighs 28.50 grams with, and 25.50 grams without, the fluid of the follicles. Neither ovary shows any enlarging Graafian follicle, nor any follicle which is approaching the hilum of the ovary, as occurs in all cases in which a follicle is about to rupture (Seaborn and Champy, '23).

Mare 459—killed on the first day of oestrus

The uterine mucosa is covered by a small amount of nearly colorless fluid, which under the microscope shows no blood cells or other formed elements. When the uterus is reversed, the folds do not hang down of their own weight, but they are more voluminous than in mares 404 and 398. The mucosa is rosy in color. The main characteristics, under the microscope, are: degeneration in the cells of the superficial epithelium, collagenous degeneration of the stroma, and increase in the number of lymphocytes.

The left ovary contains four small Graafian follicle cysts and a very large follicle which is on the point of rupturing. An exceedingly thin membrane marks the point of imminent rupture. The ovary weighed 126 grams and, after evacuation of the cysts, 80 grams. The fluid of the mature Graafian follicle weighed 39 grams. On microscopic examination the walls of the large follicle showed lutein transformation of the cells of the theca interna and of the membrana granulosa.

The right ovary weighed 70 grams before, and 65 grams after sectioning. It contained five small cysts and a degenerated corpus luteum.

Mare 51—killed on the third day of oestrus

The uterine folds are most voluminous, are semigelatinous, and show collections of a yellow fluid under the surface. There is a small amount of fluid free in the uterus, but this fluid does not contain blood cells or other formed elements. On section the mucosa 'flows,' and the blood vessels and ducts of the glands are visible with the naked eye. On reversing the uterus, the folds hang down of their own weight.

The main microscopic characteristics are: the irregularity of the epithelial cells, increase in degeneration of the stroma, and great increase in the number of lymphocytes.

The left ovary shows a large Graafian follicle which has just ruptured and from which fluid can be expressed in a small stream. The point of rupture is partially occluded by a minute blood clot. The ovary weighed 132 grams, and decreased to 75 grams upon evacuation of the cysts.

The right ovary weighed 124 grams and, after evacuation of the cysts, 72 grams. It contained several large cysts and a corpus luteum in regression.

Mare 468—killed on the second day following oestrus

The uterus shows less moisture and is not as gelatinous; when reversed, the folds hang down much less than in no. 51, of the third day of oestrus. In the cut edges the blood vessels and ducts of the glands can be indistinctly made out.

The main microscopical characteristics are: lessening of the proportion of stroma to gland tissue, decrease in degeneration of the stroma as shown by diminution of the collagenous tissue, and reduction in the number of lymphocytes.

The left ovary weighed 120 grams, contained eight cysts each the size of a cherry, and a corpus luteum 2 cm. by 3 cm., which consisted of an outer zone of a mottled dark plum color, with blood vessels coursing through it, and an inner zone of a purplish-red color and a consistency of jelly.

The right ovary contained several moderate-sized cysts. It weighed 101 grams and, after sectioning, 75 grams.

Mare 416, killed on the eighth day following oestrus

The uterus does not show the yellow color of no. 51, of the third day of oestrus, but is bluish, resembling no. 404, showing the condition of rest, of the fourteenth day following oestrus. It contains no free fluid. When the uterus is reversed, the folds hang down of their own weight for a short distance only.

The main microscopic characteristics are: continued lessening of the proportion of stroma to gland tissue; the more regular form of the cells of the surface epithelium and of the glands, and reduction in the lymphocytes.

The left ovary weighed 62 grams and, after sectioning, 55 grams. It contained a few small cysts.

The right ovary weighed 112 grams and contained several cysts and a corpus luteum showing an outer zone of a dark plum color, and an inner zone of a light yellow color and the consistency of jelly. After evacuation of the cysts, the ovary weighed 80 grams.

SURVEY OF THE OESTROUS CYCLE

A. Uterus

Surveying the uterine mucosa of the entire oestrous cycle of the mare, we find in the rest period a relatively thin bluish membrane, which upon the onset of prooestrus gradually changes to rosy color, becomes moister, and shows thickening and increase in size of the folds. As the three-day period of oestrus wears on, the much-thickened mucosa becomes yellowish in color, with heavy, wet, and flabby folds. The third day of oestrus having been left behind, the mucosa returns by degrees to normal, there being evident a thinning and dehydration of the mucosa, a shrinking of the folds, a reduction in size of the blood vessels and gland ducts, with a change in the general appearance from pink to bluish.

The uterine mucosa shows microscopically, in the prooestrus stage, an awakening growth activity in the epithelial cells manifesting itself in numerous mitotic figures. This proliferation is followed by degeneration of the epithelial cells in the oestrous period, with gradual return to normal following this. As prooestrus progresses, there is considerable collagenous degeneration in the stroma, especially superficially, which is even more pronounced in oestrus, and which gradually gives way to the normal condition in the metoestruum.

Lymphocytes, scanty in rest, become progressively more numerous until the end of oestrus, when their number is gradually reduced following this stage.

B. Ovaries

Briefly considering the changes in the ovaries which are correlated with the above-described uterine phenomena, we find during oestrus a considerable increase in the weight of these organs, this being due to the augmentation of fluid in the ripening follicle and hypertrophy of ovarian tissue. Rupture of the single ripened follicle occurs during the second or third day of oestrus; since this is the case, it is evident that the corpus luteum arising from this follicle cannot have initiated the prooestrual and oestrual uterine changes immediately preceding the rupture, nor can it be held accountable for the sexual behavior of the animal during these stages. All of these phenomena would seem more probably to be related to a hormone of the liquor folliculi or the follicular epithelium, as found by Allen et al. to be the case in spayed mice and rats injected with extract from the liquor folliculi of the pig. Champy and I ('23) brought about the occurrence of rut in unspayed rabbits by injecting into them liquor folliculi from ripe follicles of the mare. In this connection it was noted that in the mare's ripe follicles lutein transformation of the cells of the theca interna and of the membrana granulosa was found as early as the first day of oestrus. Lutein cells disappear from the corpus luteum when the uterus returns to rest.

FUNDAMENTAL IDENTITY OF THE OESTROUS CYCLE IN THE VARIOUS MAMMALS

It would appear, from a review of some of the most outstanding contributions to the subject, that the cycle of oestrus in the mare is essentially the same as that in the other mammals. Stockard and Papanicolaou for the Guinea-pig, Marshall and Robinson for the ferret, Givkovitch for the sow, Hill and O'Donoghue for Dasyurus viverrinus, Van der Stricht, Van Beneden, Courrier, Benecke, and Eimen for the bat, Heape for the monkey, and Schroeder for man, together with many others, have recorded their observations, and although they use different names for the various stages of the cycle, and although the cycle shows wide individual variations, as in such animals as the bat and the Guinea-pig, yet the fundamental ground-plan is the same throughout the mammals, as shown by a careful perusal of their gross and microscopic findings, coupled with the consideration of the sexual behavior of the animals studied. Most interesting of all, perhaps, is the cycle in the human female, which has been a subject for speculation for so many ages and about which so many erroneous ideas have gained credence. Although there are some features of it which are obscure when interpreted with reference to the cycle in other mammals, yet it seems evident that the stages which have been described in the mare are all represented in the woman and in the same order. Thus the oestrous cycle of the lower animals is equivalent to the menstrual cycle in women. This view is in line with the statement of Corner in his recent excellent review of oestrus, ovulation, and menstruation (p. 479): ''it may fairly be stated that the trend of recent work is to suggest the basic similarity of the primate reproductive cycle to that of the other mammalia.'' It seems probable that oestrus of the mare includes more than three periods of the cycle in woman, according to Schroeder's terminology—'middle of interval,' 'end of interval,' 'beginning of pre-menstruum,' and part of 'end of pre-menstruum.' This would push menstruation proper into the metoestruum of the mare.

FERTILIZATION

It has been noted that the signs of 'heat' last some six days, but that during the first three days, the prooestrous stage, the mare does not accept the male willingly. Ordinarily, if a male be available, the mare is covered as soon as oestrus proper begins. Rupture of the Graafian follicle, as noted, occurs during the second or third day of oestrus proper—thus it is a day or so after the usual time of copulation. The distance from the external os uteri to the ampulla of the Fallopian tube is some 48 cm., and it seems probable that the spermatozoa would take one or two days to travel this distance. Thus, in the ordinary course of events, the spermatozoa and ovum would arrive in the ampulla at about the same time.

PREGNANCY

If the ovum lives, i.e., if it is impregnated, the mucoid degeneration and swelling of the stroma continue and the proportion of stroma to gland tissue becomes very much greater. This increase of degeneration was shown in mare no. 32, killed on the fourth day following her oestrus. She had been covered twice during the oestrus. If the ovum dies, however, the degenerated stroma is gradually absorbed, the lymphocytes disappear, and the uterus in time returns to a condition of comparative rest.

SUPRARENALS

As to the changes in other organs of the mare during the oestrous cycle, nothing will be said here except to remark that the suprarenal glands in some cases increase in weight during the oestrous period by as much as 100 per cent. Macroscopically, the glands show an increase of the yellow material which forms the peculiar rays of the cortex. Microscopically, the principal changes consist in an increase of the spongiocytic tissue of the cortex. This tissue is probably formed by the dissolving of the labile fats by the reagents employed in the preparation of the specimen.

CONCLUSIONS

1. The oestrous cycle in the mare lasts twenty-four days and can be divided into four periods: rest—about eight days; prooestrus—three days; oestrus—three days, and metoestrus—about ten days.

2. 'Heat' lasts six days, and in its first half, the prooestrous stage, although the signs are well marked, there is an unwillingness to accept the male; in the second half, or oestrus proper, the mucous discharge from the vulva has diminished and the mare is willing to accept the male.

3. In heat there are well-marked uterine changes. In the prooestruum the mucosa becomes hypertrophied, there being mucoid degeneration of the stroma, multiplication of the epithelial cells, and augmentation of the lymphocyte content. In the oestruum these changes are very outspoken, and on the second and third days, synchronous with the rupture of the Graafian follicle, the mucosa is very thick, wet, and yellowish, with heavy, flabby, and loose folds. Blood vessels and gland ducts are seen with the naked eye and there is much degeneration of the epithelial structure and of the stroma. These changes appear to be in the nature of a preparation of the uterus for the reception of the fertilized ovum.

4. Correlated with these uterine phenomena we find an increase in weight of the ovaries in heat, the ripening of a follicle, and, at the middle or end of oestrus, ovulation. The suprarenals may double their weight in some cases.

5. Since the corpus luteum is not found until after oestrus is well advanced and degenerates before the next oestrus begins, it is probably not concerned with the initiation of oestrus. The uterine changes and sexual behavior assocciated with heat would seem more probably to be an effect of the influence of the maturing Graafian follicle.

6. Since ovulation does not occur until the middle or end of oestrus proper, the probability of fecundation is increased, doubtless, if the mare is covered during the first day that she is willing to accept the male, for this allows time for the sperm to make their way up the uterus and tube.

7. The oestrous cycle in the mare is fundamentally identical with that in other mammals and also with the menstrual cycle of woman.

In conclusion, I wish to extend thanks to Institut Pasteur, University of Paris, St. George's Hospital of London, and University of Western Ontario.

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SIMPLE FORMULAE FOR ESTIMATING THE AGE AND SIZE OF HUMAN EMBRYOS[1]

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There are several formulae of use in computing the age and size of human embryos. Some are complicated (Henry and Bastien, '04; Scammon, '21); most are limited to the determination of the age or size from one particular type of data; none cover both age and size, utilizing crown-rump or crown-heel measures.

In 1923, Scammon and Calkins published empirical formulae for calculating the age and size of fetuses between three and ten months in terms of crown-heel values. Further simplification produced the following equations:

$$\text{Age} = \left(\frac{\text{CH}}{30} + 1.49 \right)^2$$

$$\text{CH} = 30 \sqrt{\text{Age}} - 44.7$$

These formulae are said to be correct between limiting deviations of + 2.97 and — 2.66 per cent. It appears that the total body lengths used were derived by interpolation with the view of correcting for a difference of ten days between the menstrual and actual age; since, however, this correction had already been made by Mall ('10), whose data were employed, the accuracy of the results are correspondingly impaired.

Noback ('22) has also shown how to derive crown-rump or crown-heel size from the complementary data:

$$\text{CH} = \frac{3\ \text{CR} - 3}{2}$$

$$\text{CR} = \frac{2\ \text{CH} + 3}{3}$$

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[1] Contribution no. 110.

It is evident that besides exact formulae for the accurate calculation of fetal age and size there is need also of simpler ones that give reasonable approximations, quickly estimated. It is often necessary while reading, viewing specimens, or considering cases to translate age into size, or the reverse, and since some workers employ only crown-rump or crown-heel measurements, while others use both, multiplicities are introduced for which provision must be made. The formula-problem thereby resolves itself into a fulfilling of the following conditions:

1. The formulae should be simple and easily remembered.
2. They should be solvable by mental computation.
3. They should be reasonably accurate.
4. They should utilize both CH and CR size data.

A familiar rule is that of Hasse ('75), which states that for the first five fetal months the total length in centimeters equals the square of the month, whereas in the last five months it equals the month multiplied by five. As seen from the appended tabulation (table 1), this rule conforms to the requirements of simplicity and reasonable accuracy and is easily remembered. On the contrary, it has the disadvantages of fitting only CH data and of requiring a preliminary computation in estimating age to determine whether division by five or the extraction of square root is necessary.

A comparable table can be prepared for crown-rump values (table 2). Similar criticisms apply to it as to Hasse's rule. In this instance crown-rump size in centimeters is derived by multiplying the first three fetal months by 0.5, 1.5, and 2.5, respectively, and the remaining seven months by 3.5.

Attempts to construct age formulae which fulfill all of the four conditions previously enumerated have resulted in the following rules:

Standing height (in cm.) × 0.2 = age (in months)
Sitting height (in cm.) × 0.3 = age (in months)

For embryos less than 10 cm. long, that is, those of the first three months, the values thus obtained are too small,

TABLE 1

Estimation of fetal age and size based on crown-heel lengths (Hasse)

	ESTIMATED BODY LENGTH	STANDARD BODY LENGTH	DEVIATION OF ESTIMATE	PERCENTAGE DEVIATION OF ESTIMATE
mos.	*cm.*	*cm.*		
1 ×1=	1	0.25	+0.75	+300.0[2]
2 ×2=	4	3.0	+1.0	+ 33.3
3 ×3=	9	9.8	−0.8	− 8.2
4 ×4=	16	18.0	−2.0	− 11.1
5 ×5=	25	25.0	0.0	0.0
6 ×5=	30	31.5	−1.5	− 4.8
7 ×5=	35	37.1	−2.1	− 5.7
8 ×5=	40	42.5	−2.5	− 5.9
9 ×5=	45	47.0	−2.0	− 4.3
10[1]×5=	50	50.0	0.0	0.0
		Sum	12.7	73.3
		Mean	1.3	7.3

[1] The actual fetal age at full term is 270 days, or 9.6 months; the corresponding estimated size is 48 cm., which is less correct than that recorded.

[2] Although the actual error is low, the percentage error is high and unrepresentative. For this reason it is omitted from the sum and mean for the column.

TABLE 2

Estimation of fetal age and size based on crown-rump lengths

	ESTIMATED BODY LENGTH	STANDARD BODY LENGTH	DEVIATION OF ESTIMATE	PERCENTAGE DEVIATION OF ESTIMATE
mos.	*cm.*	*cm.*		
1 ×0.5=	0.5	0.25	+0.25	+100.0[2]
2 ×1.5=	3.0	2.5	+0.5	+ 20.0
3 ×2.5=	7.5	6.8	+0.7	+ 10.3
4 ×3.5=	14.0	12.1	+1.9	+ 15.7
5 ×3.5=	17.5	16.7	+0.8	+ 4.8
6 ×3.5=	21.0	21.0	0.0	0.0
7 ×3.5=	24.5	24.5	0.0	0.0
8 ×3.5=	28.0	28.4	−0.4	− 1.4
9 ×3.5=	31.5	31.6	−0.1	− 0.3
10[1]×3.5=	35.0	33.6	+1.4	+ 4.2
		Sum	6.1	56.7
		Mean	0.6	5.7

[1] If the real age of 9.6 months at full term were used, the estimated value would be 33.7 cm., which agrees closely with the standard size of 33.6 cm.

[2] Omitted from calculation of the sum and mean for the column. (See footnote 2, table 1).

since in such early embryos the growth curve is of a different contour. This discrepancy, however, can be corrected with fair success by adding one month to the results gained by direct multiplication; obviously, there will be a certain incongruity in the results bordering the correction point thus arbitrarily set at 10 cm. These values, with the actual and percentage deviation from normal, are collected in the subjoined tabulations (tables 3 and 4).

It is at least of passing interest to point out that rather accurate results may be obtained for the last five lunar months by replacing 0.2 and 0.3 as the multiplying factor by 0.19 and 0.29. The figures thus obtained are collected in tables 5 and 6.

In a similar fashion, adherence to the four fundamental stipulations was attempted in educing rules which calculate size from age:

Age (in months) ÷ 0.2 = standing height (in cm.)
Age (in months) ÷ 0.3 = sitting height (in cm.)

Again the results are out of line for embryos of the first three months, but reasonable correction is obtained when 4 cm. are subtracted from the values obtained directly by division. These determinations are assembled in tables 7 and 8.

As before, quite exact values are obtainable for the last five lunar months if 0.19 and 0.29 replace 0.2 and 0.3 as divisors (tables 9 and 10).

Those who are interested in mnemonic aids will perceive that all four formulae deal only with the digits, one, two, three, four, and their numerical sum which is ten. The use

$$\begin{array}{c} 1 \\ 2 \\ 3 \\ 4 \\ \hline 10 \end{array}$$

of the appropriate multiplying and dividing factors, 0.2 and 0.3, is apparent in each case, for obviously the higher factor (0.3) always accompanies the shorter fetal length (CR), and vice versa. The digit 1 must be the correction for age and 4 for length—the reverse is patently absurd. Embryos less than 10 cm. long, or three months old, are the ones for which

TABLE 3

Estimation of fetal age from crown-heel length

STANDARD LENGTH	ESTIMATED AGE	CORRECT AGE	DEVIATION OF ESTIMATE	PERCENTAGE DEVIATION OF ESTIMATE
cm.	*months*	*months*		
0.25×0.2=	.05+1=1.05	1.0	+0.05	+ 5.0
3.0 ×0.2=	.60+1=1.6	2.0	−0.40	−20.0
9.8 ×0.2=	1.96+1=2.96	3.0	−0.04	− 1.3
18.0 ×0.2=	3.60	4.0	−0.40	−10.0
25.0 ×0.2=	5.00	5.0	0.00	0.0
31.5 ×0.2=	6.30	6.0	+0.30	+ 5.0
37.0 ×0.2=	7.40	7.0	+0.40	+ 5.7
42.5 ×0.2=	8.50	8.0	+0.50	+ 6.3
47.0 ×0.2=	9.40	9.0	+0.40	+ 4.4
50.0 ×0.2=	10.00	10.0[1]	0.00	0.0
		Sum	2.85	57.7
		Mean	0.29	5.8

[1] Exact full-term age is 9.6 lunar months.

TABLE 4

Estimation of fetal age from crown-rump length

STANDARD LENGTH	ESTIMATED AGE	CORRECT AGE	DEVIATION OF ESTIMATE	PERCENTAGE DEVIATION OF ESTIMATE
cm.	*months*	*months*		
0.25×0.3=	.075+1=1.075	1.0	+ .075	+ 7.5
2.5 ×0.3=	.75 +1=1.75	2.0	− .25	−12.5
6.8 ×0.3=	2.04 +1=3.04	3.0	+ .04	+ 1.3
12.1 ×0.3=	3.63	4.0	− .37	− 9.3
16.7 ×0.3=	5.01	5.0	+ .01	+ 0.2
21.0 ×0.3=	6.30	6.0	+ .30	+ 5.0
24.5 ×0.3=	7.35	7.0	+ .35	+ 5.0
28.4 ×0.3=	8.52	8.0	+ .52	+ 6.5
31.6 ×0.3=	9.48	9.0	+ .48	+ 5.3
33.6 ×0.3=	10.08	10.0[1]	+ .08	+ 5.0
		Sum	2.48	53.6
		Mean	0.25	5.4

[1] Exact full-term age is 9.6 lunar months.

corrections are made. With these numbers (1, 2, 3, 4, 10) once in mind, it is easy to reconstruct the formulae at any time by using them as common sense dictates.

TABLE 5

Exact calculation of later fetal age from crown-heel length

STANDARD LENGTH	ESTIMATED AGE	CORRECT AGE	DEVIATION OF ESTIMATE	PERCENTAGE DEVIATION OF ESTIMATE
cm.	*months*	*months*		
31.5×0.19=	5.99	6.00	—0.01	—0.2
37.0×0.19=	7.03	7.00	+0.03	+0.4
42.5×0.19=	8.08	8.00	+0.08	+1.0
47.0×0.19=	8.93	9.00	—0.07	—0.8
50.0×0.19=	9.50	9.64	—0.14	—1.4
		Sum	0.33	3.8
		Mean	0.07	0.8

TABLE 6

Exact calculation of later fetal age from crown-rump length

STANDARD LENGTH	ESTIMATED AGE	CORRECT AGE	DEVIATION OF ESTIMATE	PERCENTAGE DEVIATION OF ESTIMATE
cm.	*months*	*months*		
21.0×0.29=	6.09	6.00	+0.09	+1.5
24.5×0.29=	7.12	7.00	+0.12	+1.7
28.4×0.29=	8.24	8.00	+0.24	+3.0
31.6×0.29=	9.16	9.00	+0.16	+1.8
33.6×0.29=	9.74	9.64	+0.10	+1.0
		Sum	0.71	9.0
		Mean	0.14	1.8

SUMMARY

Formulae for estimating the age and size of human embryos and fetuses have been developed which are simple, reasonably accurate, and utilize both crown-rump and crown-heel data:

Standing height (cm.) × 0.2 = age (months)
Sitting height (cm.) × 0.3 = age (months)
(For embryos less than 10 cm., add one month)

Age (months) ÷ 0.2 = standing height (cm.)
Age (months) ÷ 0.3 = sitting height (cm.)
(For embryos of first three months, subtract 4 cm.)

TABLE 7

Estimation of fetal size in crown-heel measure

	ESTIMATED BODY LENGTH	STANDARD BODY LENGTH	DEVIATION OF ESTIMATE	PERCENTAGE DEVIATION OF ESTIMATE
mos.	*cm.*	*cm.*		
1 ÷0.2=	5.0—4.0= 1	.25	+ 0.75	+300.0[2]
2 ÷0.2=	10.0—4.0= 6	3.0	+ 3.00	+100.0[2]
3 ÷0.2=	15.0—4.0=11	9.8	+ 1.20	+ 12.2
4 ÷0.2=	20.0	18.0	+ 2.0	+ 11.1
5 ÷0.2=	25.0	25.0	0.0	0.0
6 ÷0.2=	30.0	31.5	— 1.5	— 4.8
7 ÷0.2=	35.0	37.1	— 2.1	— 5.7
8 ÷0.2=	40.0	42.5	— 2.5	— 5.9
9 ÷0.2=	45.0	47.0	— 2.0	— 4.3
10[1]÷0.2=	50.0	50.0	0.0	0.0
		Sum	15.1	44.0
		Mean	1.5	4.4

[1] If the exact age of 9.64 months at full term were used, the estimated size would be 48 cm.

[2] Omitted from the calculation of the sum and mean for the column. (See footnote 2, table 1).

TABLE 8

Estimation of fetal size in crown-rump measure

	ESTIMATED BODY LENGTH	STANDARD BODY LENGTH	DEVIATION OF ESTIMATE	PERCENTAGE DEVIATION OF ESTIMATE
mos.	*cm.*	*cm.*		
1 ÷0.3=	3.3—4=—0.7	0.25	—0.95	—380.0[2]
2 ÷0.3=	6.7—4= 2.7	2.5	+0.2	+ 8.0
3 ÷0.3=	10.0—4= 6.0	6.8	—0.8	— 11.8
4 ÷0.3=	13.3	12.1	+1.1	+ 9.1
5 ÷0.3=	16.7	16.7	0.0	0.0
6 ÷0.3=	20.0	21.0	—1.0	— 4.8
7 ÷0.3=	23.3	24.5	—1.2	— 4.9
8 ÷0.3=	26.7	28.4	—1.7	— 6.0
9 ÷0.3=	30.0	31.6	—1.6	— 5.1
10[1]÷0.3=	33.3	33.6	—0.3	— 0.9
		Sum	8.9	50.6
		Mean	0.89	5.1

[1] If the exact age of 9.6 months at full term were used, the estimated size would be 32 cm.

[2] Omitted from the calculation of the sum and mean for the column. (See footnote 2, table 1).

TABLE 9

Exact calculation of later fetal size in crown-heel measure

	ESTIMATED BODY LENGTH	STANDARD BODY LENGTH	DEVIATION OF ESTIMATE	PERCENTAGE DEVIATION OF ESTIMATE
mos.	*cm.*	*cm.*		
6.00÷0.19=	31.6	31.5	+0.1	+0.3
7.00÷0.19=	36.8	37.1	—0.3	—0.8
8.00÷0.19=	42.1	42.5	—0.4	—0.9
9.00÷0.19=	47.4	47.0	+0.4	+0.9
9.64÷0.19=	50.7	50.0	+0.7	+1.4
		Sum	0.19	4.2
		Mean	0.04	0.9

TABLE 10

Exact calculation of later fetal size in crown-rump measure

	ESTIMATED BODY LENGTH	STANDARD BODY LENGTH	DEVIATION OF ESTIMATE	PERCENTAGE DEVIATION OF ESTIMATE
mos.	*cm.*	*cm.*		
6.00÷0.29=	20.7	21.0	—0.3	— 2.9
7.00÷0.29=	24.1	24.5	—0.4	— 1.6
8.00÷0.29=	27.6	28.4	—0.8	— 2.8
9.00÷0.29=	31.0	31.6	—0.6	— 1.9
9.64÷0.29=	33.2	33.6	—0.4	— 1.2
		Sum	2.5	10.4
		Mean	0.5	2.1

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SPINAL-CORD INJURY AND NERVE-CELL PROLIFERATION IN THE EMBRYO

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The number of nerve cells which develop within certain regions of the spinal cord of amphibians is not inherently fixed. This fact has abundant experimental proof(1, 2, 3). In one series of experiments dealing with this question, a unit of cord comprising the seventh, eighth, and ninth segments was substituted for the brachial region (third, fourth, and fifth segments). Pieces so grafted developed more nerve cells in the new situation than in their original one, and the number produced was found to correspond very closely to that normally proliferating within the brachial region.

A study of cellular development under normal and experimental conditions has led to the view that the final production of cells which characterizes certain regions of the cord may be influenced largely by stimuli reaching such regions through the invasion of axones from higher centers possessed of extensive inherent capacity for neuronic development. It was suggested in this connection that the cellular hyperplasia observed in the seventh, eighth, and ninth segments of the cord, when substituted for the third, fourth, and fifth, may be an expression of such an influence, particularly since cellular proliferation within each grafted segment was found to correspond closely to that typifying the respective brachial segment which has been supplanted (table 1). The fact that more nerve cells develop at the cephalic end of the brachial region than at the caudal has been looked upon as indicating that perhaps a greater number of longitudinal projection fibers terminate within the former than in the latter region.

The greater number of cells developing within the brachial region as compared with the number occurring in segments caudal thereto cannot be ascribed directly to the activities of the limb(4).

Coghill(5) has shown in a most thorough investigation that, in early stages of nervous development in Amblystoma (non-motile, early flexure, coil, and early swimming stages), there are localized regions where differentiation and proliferation of cells take place according to a very definite pattern, and that acceleration of these processes is definitely related to the development of the behavior pattern. He further shows that one process tends to become accelerated where the other is retarded, and that both take place in the form of waves which probably pass cephalocaudad through the brain and spinal cord. His figure 8 shows that, during the non-motile and early flexure stages, neuroblastic proliferation is higher throughout the anterior limb region of the cord than in segments cephalad or caudad to this portion, though there is as yet no nervous connection with the limb buds. An examination of his figure shows further that at a later period (early swimming) the neuroblastic proliferation decreases rapidly in successive levels through the brachial region. His data indicate clearly that in different parts of the central nervous system there are regions possessed of high potentiality for proliferation and that relative rates vary within these regions according to the stage of development.

Coghill's observations afford convincing evidence that in early stages of development the resolution of indifferent cells into neuroblasts, the early proliferation of these neuroblasts, and the regional localization in the acceleration and retardation of these processes are accomplished under influences which precede the activating stimuli coming from ingrowing nerve roots and tracts. These more elementary influences seem to be bound up with the hereditary organization of the protoplasm and the primary physiological gradients.

In referring to the cellular hyperplasia which was observed in the transplanted spinal segments described in my original spinal-cord experiments(1), Coghill(5, p. 103) suggests that injury inflicted during the grafting has played some part in the results observed on subsequent proliferation. He suggests this because of the fact that in several instances (according to him) where the seventh, eighth, and ninth segments were transplanted into the brachial region (third, fourth, and fifth segments), the number of cells in the various transplanted segments was found to be higher than in the corresponding normal segments from the limb region (table 1). When an analysis is made of this table it is readily seen that the total number counted in the transplanted segments as compared with the total number in the third, fourth, and fifth segments from the control animal shows an increase of 138 cells above the normal in only one case (case TrSc 139) whereas in the other (case TrSc 137) the total number shows a decrease of 376 cells below the normal. Coghill also calls attention to the fact that proliferation within the seventh, eighth, and ninth segments of the cord in the operated cases is greater than in the corresponding segments from a normal

TABLE 1

Showing number of cells counted in twenty consecutive transverse sections of the right half of the spinal cord at the levels of the third, fourth, and fifth segmental nerves, respectively (anterior limb level)

CONDITIONS	CASES	III NERVE LEVEL	IV NERVE LEVEL	V NERVE LEVEL
Normal	AS4	3464	2796	2346
Seventh, eighth, and ninth spinal segments substituted for the third, fourth, and fifth, respectively	TrSc 137[1]	3130	2600	2500
	TrSc 139[1]	3043	2973	2728
Anterior limb level of spinal cord (third, fourth, and fifth segments) reversed end for end	ReSc 1[2]	3500	2593	2215
	ReSc 10[2]	3265	2809	2326

[1] In case TrSc 137 and TrSc 139, the third, fourth, and fifth nerve levels represent, respectively, the transplanted seventh, eighth, and ninth.

[2] In cases ReSc 1 and ReSc 10, the third nerve level represents the reversed fifth, whereas the fifth nerve level represents the reversed third.

animal, and makes use of this difference as a point favoring the idea that some factor other than fiber ingrowth is affecting the final results, and which he later suggests as being the effect of injury. Attention is here called to the fact, however, that the seventh, eighth, and ninth segments in the operated cases were never disturbed. The experiments consisted in excising the third, fourth, and fifth segments and replacing them by the seventh, eighth, and ninth from another embryo. The latter segments in the host have been used as a normal control, hence injury could not possibly have played a part in the slight cellular increases observed in these segments. Cell counting on so large a scale is only approximate at the most, and the small differences to which Coghill refers are probably insignificant and must be looked upon as slight errors due to the difficulty of making absolutely accurate counts.

In connection with the question of the effects of injury on proliferation, the experiments dealing with the reversals of the anterior limb region of the spinal cord are instructive(2). Here the third segment has replaced the fifth, and the latter has been placed in the position of the normal third. Reference to table 1 will show that the number of cells counted in the transplanted third segment in both cases was slightly lower than in the normal fifth, and in the transplanted fifth segment the number in case ReSc 10 (table 1) is approximately 200 less than in the normal third. From the fact that, in three instances out of four transplanted segments, the proliferation is slightly less than that characterizing the normal, injury must be looked upon as being an ineffectual factor in the results.

In experiments involving the transplantation of the medulla and the anterior end of the spinal cord(3), proliferation throughout the medulla was decidedly decreased over that in the normal medulla, whereas in the spinal portion of the graft lying caudal to the medulla, proliferation was increased over the normal. Since both parts of the graft were subject to the same injury, it is difficult to imagine any significant effects due to this factor.

Hooker(6) has studied the healing processes in transected spinal cords of Amblystoma and Rana. He showed a rapid proliferation of cells in the vicinity of the wound seven days after the operation. In a later work(7) he reports that injuries to the cord produce proliferation by mitosis of cells of the mantle layer. Coghill has suggested, on the basis of Hooker's observations, that injury inflicted on the cord has had some part in my results. Even if increased cellular mitoses do occur as repair processes in response to injury, such processes seem to be ineffectual in changing the final quantity of cells produced.

Abnormal proliferation following injury does come about when the external limiting membrane fails to repair. No doubt this membrane plays an important rôle in this connection and also in determining the orientation of the neurones. Hoadley(8) has shown, in pieces of chick mesencephalon which were grafted along with somitic tissue to the chorio-allantoic membrane in the chick, that nerve fibers which normally act as visual correlation tracts in the mesencephalon leave the graft and grow into the differentiating muscle.

Nicholas(9) describes a cellular proliferation in the cord following implantation of a limb into the middorsal line of the embryo. There seems to be no reason to regard this proliferation as a normal morphogenetic reaction. There has been no evidence forthcoming from my own limb experiments to indicate any intraspinal hyperplasia in response to the functional demands of a heterotopic limb. Neither does injury of the cord result as a rule in any such striking cellular increases. The type of proliferation which Nicholas describes appears to follow as a result of extensive injury to the external limiting membrane, and the condition is similar to abnormal or pathological responses met with in a limited number of cases following interchanging of spinal-cord segments. The atypical cellular reaction reminds one of abnormal bone proliferation following denudation by the periosteum, and must be looked upon as a pathological condition.

Coghill(5) shows (his figs. 7 and 8) that in early stages, cell proliferation and differentiation are associated in the growth of the spinal cord. He finds that the seventh, eighth, and ninth segments, which I transplanted into the brachial region and which underwent marked hyperplasia, fall well within the range of relatively high potentiality of both proliferation and differentiation. Since the tenth, eleventh, and twelfth segments fall beyond the limits of so great a potentiality, Coghill raises the question as to whether these latter segments would have the same capacity for growth in the brachial region as did the seventh, eighth, and ninth segments. This is an interesting point and experiments to test this matter will be carried out. By comparing proliferation within such segments with that in the seventh, eighth, and ninth, both under normal conditions and when transplanted into the brachial region, greater possibilities are afforded to determine more definitely than hitherto the extent to which various factors influence cellular development.

SUMMARY

Increased cellular mitosis resulting from injury to the spinal cord in the embryo as observed by Hooker(6, 7), and to which Coghill(5) refers as playing a part in the results of my former spinal-cord-transplantation experiments(1), appears to have no effect on the final number of cells produced. Cellular hyperplasias, therefore, which ensue in segments of the cord from the midtrunk region when transplanted into the brachial region (table 1) must be looked upon as resulting from influences other than those brought about through injury.

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ON THE CYTOPLASMIC INCLUSIONS IN THE MALE GERM CELLS OF CYRTOPHYLLUS

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THREE PLATES (THIRTY-FOUR FIGURES)

This line of investigation was undertaken with the purpose of making a study of the idiosome, Golgi apparatus, acrosome, middle piece, and any other structures which might be found within the cytoplasm of the cell. It is a pleasure to acknowledge my indebtedness to Dr. F. Payne, under whose direction this work was pursued.

MATERIAL AND TECHNIQUE

In examining the germ cells of several families of insects, those of some of the Locustidae (katydid) exhibited characteristics which seemed to make them most favorable for the study of cytoplasmic inclusions. The material used in this study was collected at Winona Lake, Indiana, during the summers of 1922 and 1923.

My material for the most part was fixed in Flemming, Allen's modified Bouin, Flemming without acetic, Champy's fluid, and an equal mixture of Flemming and Bouin without the acetic acid.

These fixations were followed by iron haematoxylin, safranin and licht grün, safranin and gentian violet, and Benda's alizarin and crystal violet.

The material fixed in Flemming and stained in iron haematoxylin brought out everything that was brought out by the other methods, and in addition proved to be more reliable. Except for comparison, this technique was used exclusively.

CYRTOPHYLLUS

1. Mitochondria

Mitochondria are always present in the spermatogonia. Their behavior seems less fixed and exact at this period than in later stages. They appear as an irregular mass which stains but very little darker than the cytoplasm (fig. 1, *M*).

It is impossible to demonstrate with any certainty the presence of mitochondria during the spermatogonial divisions. As the spindle fibers disappear in the telophase of the spermatogonial divisions, the mitochondria collect in the midst of the disappearing spindle (fig. 3). Their appearance here is that of a homogeneous substance which stains but very little darker than the cytoplasm. This behavior would seem to indicate that the mitochondria pass through the spermatogonial divisions in close proximity to the spindle, as has been demonstrated by Payne, Shaffer, and Lewis and Robertson.

At the beginning of the growth period the cytoplasm forms a narrow layer about the nucleus. This layer stains much darker than is usual for the cytoplasm and contains numerous dark irregular granules, the nature of which I do not know. They disappear during the growth period. The mitochondria lie as an elongated mass in the layer of darkly staining cytoplasm (fig. 4). This mass shows considerable irregularity and variation in form, but appears as a homogeneous substance. Sometimes the periphery is marked by a fine darkly staining line. There is no indication of the presence of either granules or threads. During the growth period the mass breaks up to form a number of smaller, more or less spherical bodies (fig. 5). I find no indication that the mitochondria increase in amount during the growth period, as was suggested by Lewis and Robertson ('16) and Shaffer ('17). Mitochondria are not visible in either spermatocyte division. During the telophase of the spermatocyte divisions they reappear in the region of the spindle remnant in much the same manner as was noted in the spermatogonial divisions (figs. 7, 11, 12). Following the second spermatocyte division, the mitochondria take the form of a sphere. The central mass appears as a homogeneous substance staining somewhat darker than the cytoplasm of the cell. The periphery of this sphere is marked by a darkly staining line (fig. 13). This mitochondrial mass or nebenkern undergoes a change in the spermatid (fig. 14). The body enlarges and stains more intensely in the center. The entire mass is surrounded by the dark peripheral line as before. Sometimes pseudopodia-like projections extend from the darker central mass into the surrounding clear area (fig. 14). These projections may reach the peripheral line, in which case they seem to be fused with it.

When the axial filament first appears, the nebenkern takes a position at the side of it (fig. 16). It then constricts and divides into halves. These halves, remaining in contact with each other, elongate. One end of the nebenkern extends to and seems to be in contact with the body which will become the middle piece of the spermatozoon (fig. 17). As the nebenkern continues to elongate the two halves separate and the axial filament takes a position between them (figs. 18, 19).

GOLGI APPARATUS AND IDIOSOME MATERIAL

Bowen ('22) has pointed out the fact that the Golgi apparatus and idiosomic substance together form a definite inseparable structure (the Golgi body-idiosome complex). This structure we shall for convenience refer to simply as the Golgi apparatus or Golgi body.

In material which has been fixed in strong Flemming and stained with Heidenhain's iron-haematoxylin the Golgi apparatus appears as a distinct body which stains intensely black. This body varies considerably in form in the spermatogonia. It usually appears as an elongated body of more or less irregularity in outline, the periphery having a somewhat ragged appearance (fig. 1, *G*). The Golgi apparatus is always present as a single body in the spermatogonial cell.

In the spermatogonial divisions the Golgi body divides into approximately equal parts, one part passing into each of the daughter cells. In a few cases one of these parts has been seen to subdivide into two, which later, in the telophase of the spermatogonial division, reunite into a single body.

Figure 2 shows the metaphase plate of the spermatogonial division in which the Golgi body has divided and approximately one-half has moved to each pole. Figure 4 shows a stage at the beginning of the growth period where the two smaller bodies are uniting to form again a single large Golgi body. In Cyrtophyllus this is as near as the Golgi body ever comes to breaking up during either the spermatogonial or the spermatocyte divisions. Bowen has described a number of cases in which the Golgi apparatus breaks up into small granules during mitosis, and these granules usually migrate to the spindle poles. In the spermatogonial divisions of Cyrtophyllus the Golgi body divides before the metaphase is reached. The Golgi body seems to divide into approximately equal halves, which are more or less uniform in size and shape.

At the beginning of the growth period the Golgi apparatus appears as an elongated body lying within the heavy band of cytoplasm which is characteristic of that stage. During the growth period the body shows a great deal of irregularity in both size and form. Often it appears to be larger than at any other stage and does not stain as densely black. Figure 5 shows an unusually large Golgi body lying within a clear area which is bounded by a dark line. This clear area and boundary line are not often present. In the metaphase of the spermatocyte divisions the Golgi body usually lies in the plane of the metaphase plate as a single body (figs. 6 and 8). In the spermatocyte divisions the body does not usually divide until after the chromosomes have commenced to constrict and pull apart. Figure 8 shows the Golgi body constricting and preparing to divide. It will be seen that the Golgi body often presents a ragged appearance in the first spermatocyte division as if it were made up of a mass of fibers. However, the intensity with which this body stains makes it impossible to draw any accurate conclusions as to its structure. I will discuss the structure of the Golgi bodies in a later paragraph. It is characteristic of the first spermatocyte division that the cytoplasm contains a number of lines woven into rather elaborate designs (fig. 6). These almost entirely disappear during the anaphase and I am unable to say anything as to their significance. The second spermatocyte metaphase is characterized by a number of lines of a granular nature (figs. 8, 9). These, too, disappear during the anaphase of the division.

After the Golgi body has divided, in the spermatocyte divisions, the two resulting bodies never subdivide, as was sometimes the case in the spermatogonial divisions. It always passes into the anaphase as a single body. The Golgi apparatus passes into the spermatid as an irregularly shaped body staining intensely black (fig. 12). This body soon takes the form of a more or less perfect sphere (fig. 13). The Golgi body and the mitochondria always lie close together in the spermatid.

During the stage immediately preceding the formation of the axial filament, the Golgi body begins to undergo a transformation which results in the formation of the acrosome. The Golgi body at this stage Bowen chooses to call the acroblast. This structure he points out to be the exact equivalent of the idiosome plus the Golgi apparatus of the spermatocyte. On one side of the acroblast at this stage there appears a small clear vesicle similar to that described by Bowen ('22). As this vesicle appears, the material immediately adjoining it either migrates or is used up in its formation, thus leaving a depression in the acroblast at that point (fig. 14, *a*).

As the axial filament is formed and the mitochondria elongate to form the mitochondrial sheath, the acroblast migrates upward and the vesicle becomes attached to the nucleus (fig. 17). The axial filament is at first perpendicular to the nuclear wall. The nucleus then turns in relation to the axis cylinder until the axial filament lies as a tangent to the nucleus rather than perpendicular to it (figs. 17, 20). The acroblast is attached to the side of the nucleus which lies closest to the axial filament and at a position which is approximately midway between the base and future head region of the spermatozoon (fig. 20, *A*).

After the acrosomal vesicle has attached itself to the nucleus, it enlarges and begins to free itself from the remainder of the acroblast. Following the formation of the acrosomal vesicle, still another body arises from the acroblast in much the same manner as the vesicle. This body stains more darkly than the acrosomal vesicle and retains its connection to both the vesicle and the Golgi remnant until completely formed (fig. 20). The nucleus now turns until the future longitudinal axis of the spermatozoon is in direct line with the axial filament (fig. 21). The vesicle has migrated to the anterior of the nucleus. At the same time the acrosomal vesicle, the second derivative from the acroblast, and the Golgi remnant separate from each other (fig. 21). Thus we have three separate and distinct structures forming out of the Golgi-idiosome complex (acroblast). The acrosomal vesicle retains its connection with the nucleus and forms the acrosome of the spermatozoon. I am unable to show conclusively what happens to the second body, but my observations would indicate that it passes out down the tail of the spermatozoon and is thrown off. This is without doubt the fate of the Golgi remnant (figs. 23, 24, 25).

As the sperm nucleus begins to elongate, the acrosomal vesicle flattens out against the nucleus and forms a cap at the head of the nucleus. Figure 23 shows this cap staining densely black. In figure 24 the nucleus has still further elongated. The acrosome is not flattened out as much as before and it has again assumed a clear vesicle-like appearance. As the nucleus continues to elongate and the nuclear material becomes condensed, the acrosome becomes cap-shaped and seems to be taken in as a part of the spermatozoon. The center is not as clear as before (fig. 25). The posterior wall of the vesicle seems to be losing its identity, while the anterior wall is thickening to form the head piece of the spermatozoon. This anterior thickened portion continues to enlarge. In the meantime the lower portion of the acrosome begins to stain black and to become separated from the heavy anterior cap by a light staining area (fig. 26). This posterior portion seems to move down into and to mix with the nuclear material, the anterior portion alone shaping itself into the headpiece of the spermatozoon. It is not certain whether or not both Golgi material and idiosome substance go into the acrosome. The appearance of the acrosomal vesicle would suggest that it might be made up of both Golgi material and idiosome substance as was the Golgi body of the spermatid.

The headpiece of the spermatozoon finally takes the form illustrated in figures 27, 28, and 29. Figures 28 and 29 show two views of the headpiece of the spermatozoon. Accompanying these figures are a number of cross-sections through the headpiece. The headpiece takes somewhat the shape of a ring or band, one side of which is drawn out anteriorly, while the opposite side is extended posteriorly. The anterior portion is larger than the posterior portion.

It will be seen from the above description that the Golgi body in Cyrtophyllus stains densely black in all stages of development, and so does not give any evidence as to its structure. In Amblycorypha the Golgi body goes through the spermatogonia in much the same manner, but presents a very different appearance beginning with the spermatocyte divisions. In this species I have not been able to follow the Golgi apparatus through the spermatocyte divisions. In the telophase of the spermatocyte divisions it makes its appearance near the nucleus. The body here appears as a compact spherical mass which stains with almost the same intensity as the cytoplasm and the mass is bounded by a line of darkly staining substance. The Golgi body retains this appearance throughout the remainder of its development until the formation of the acrosome. Bowen found a similar structure in which he designated the central mass as the idiosome substance and the darkly staining periphery as the Golgi rodlets.

THE MIDDLE-PIECE AND AXIAL FILAMENT

Immediately preceding the formation of the axial filament, two small granules make their appearance close to the nucleus and usually (always?) on the side next to the mitochondria.

I have not been able to trace these granules back to the centrosomes of the last spermatocyte division, but all the evidence I have at hand would indicate that these granules are the centrosomes.

The axial filament arises from one of these granules while the granules are still some distance apart and before they have come in contact with the nucleus (fig. 15). After the formation of the axial filament, the two granules come in contact with the nucleus, enlarge, elongate, and commence to fuse next to the nucleus (fig. 16). The fusion continues until the granules have formed a single compact body (fig. 17).

The middle-piece, as the spermatozoon continues to elongate, becomes a distinct more or less cone-shaped body with the large end next to the nucleus (figs. 24 to 29). It retains this form until the spermatozoon is almost completely developed. The nucleus then extends down and comes in contact with the middle-piece, and the middle-piece loses its identity.

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PLATE 1

EXPLANATION OF FIGURES

Cyrtophyllus

1 A spermatogonial cell showing mitochondria and Golgi body.

2 Spermatogonial division showing the Golgi body divided.

3 Telophase of spermatogonial division showing mitochondria collecting in region of spindle.

4 and 5 The mitochondria and Golgi body during growth period.

6 Metaphase of first spermatocyte division showing Golgi body still undivided.

7 Telophase of first spermatocyte division showing the Golgi body in each daughter cell and the mitochondria appearing in region of spindle fibers.

8 Second spermatocyte division. The Golgi body is constricting.

G, Golgi apparatus; *M*, mitochondria. × 2589.

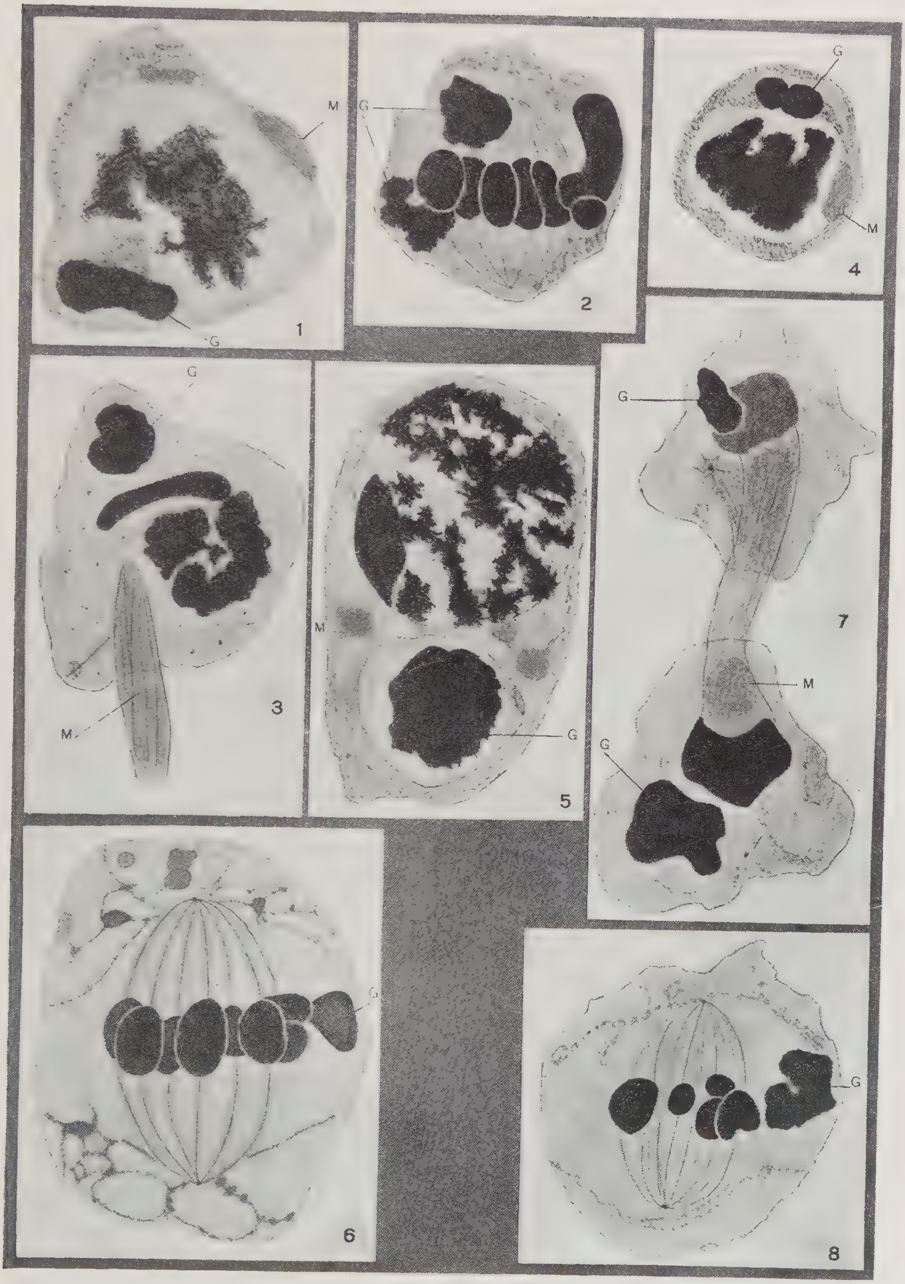
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PLATE 2

EXPLANATION OF FIGURES

Cyrtophyllus

9 to 11 The behavior of the Golgi body in the second spermatocyte division. Figure 9 shows Golgi body after division has occurred.

12 and 13 Show the reconstruction of the nucleus and the formation of the nebenkern following the second spermatocyte division.

14 Spermatid showing the nebenkern and the formation of the acrosome vesicle out of the Golgi body.

15 to 20 Stages in the transformation of the spermatid into the spermatozoon. Figure 12 shows the beginning of the axial filament. Figure 16 shows the beginning of the transformation of the granules of figure 15 into the middle-piece. Figures 18 and 19 show cross-sections through the axial filament following the stage in figure 17. Figure 20 shows the separation of the acrosome vesicle and a second body from the acroblast.

G, Golgi apparatus; *M,* mitochondria; *N,* nebenkern; *a,* acroblast; *A,* acrosome.

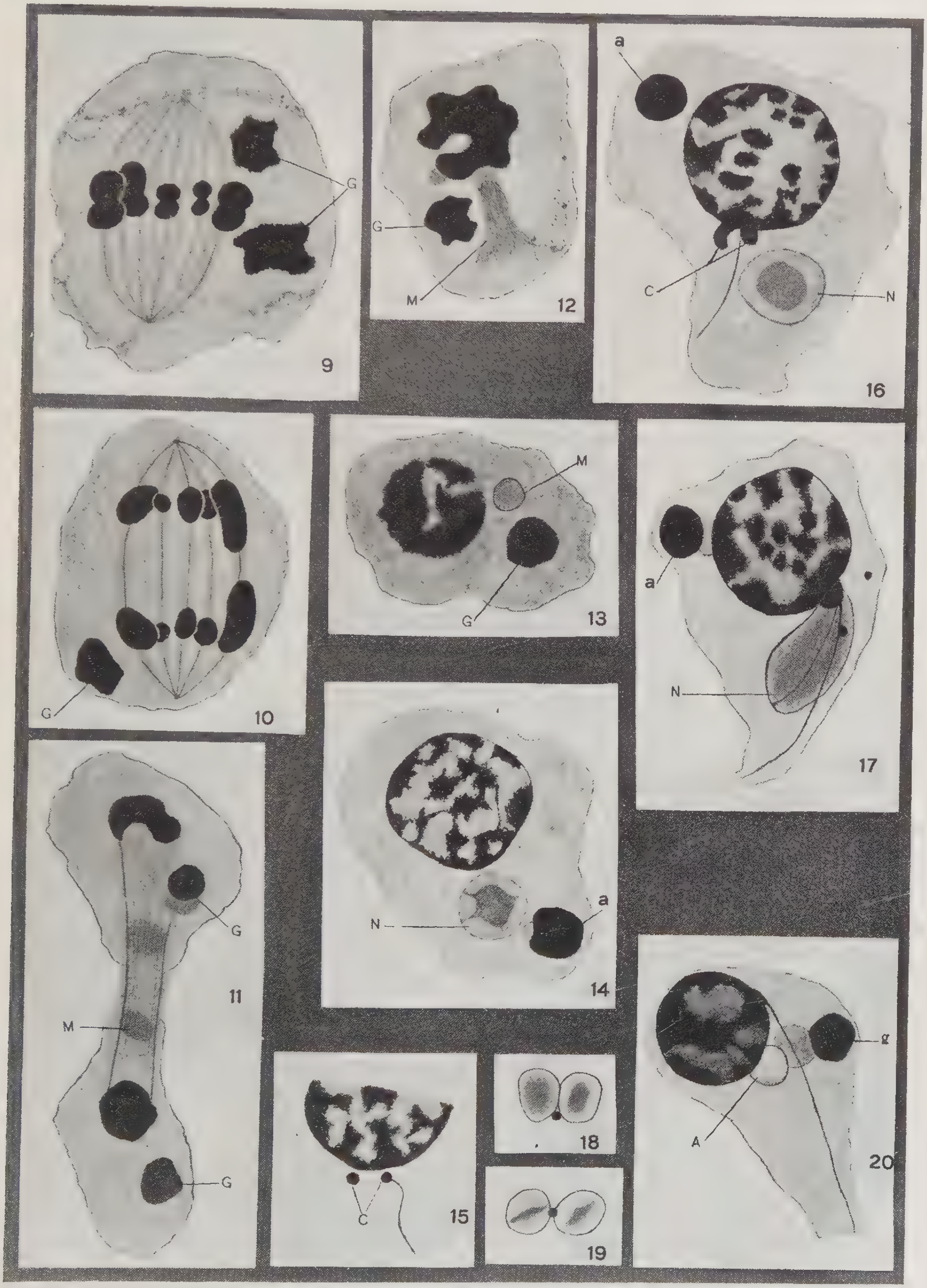
G
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PLATE 3

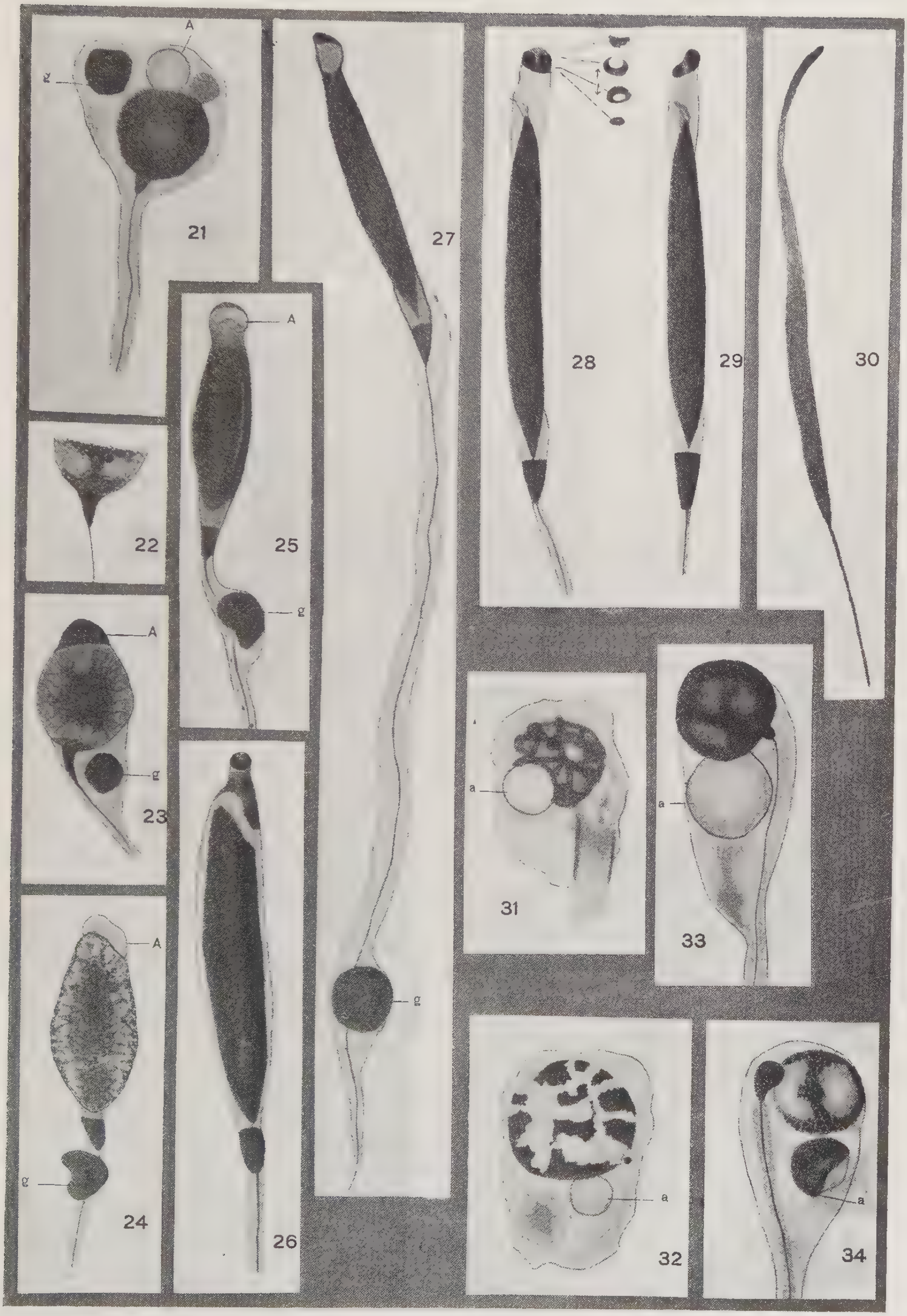
EXPLANATION OF FIGURES

Cyrtophyllus

21 to 30 Further stages in the development of the spermatozoon. In figures 22 and 23 chromatin material seems to be in contact with the middle-piece. Figures 23 to 25 show the Golgi remnant being thrown off and the stages in the transformation of the acrosome vesicle into the headpiece of the spermatozoon. Figure 26 is a further stage in the transformation of the acrosome.

31 to 33 Showing the appearance of the Golgi body in the locustid Amblycorypha.

34 Shows the appearance of the Golgi body in the locustid Scudderia, as the acrosome vesicle is forming.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THE DEVELOPMENT OF THE PRONEPHROS IN RELATION TO THE BEHAVIOR PATTERN IN AMBLYSTOMA[1]

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The descriptive part of this paper is a résumé of a rather extensive unpublished account by the junior author of the development of the pronephros and Wolffian duct in Amblystoma as correlated with the development of the behavior of the embryos as described by the senior author (Coghill).[2] Results recently published by the senior author (Coghill, '24 a, '24 b) and by his students, Faris ('24) and Moore (Coghill and Moore, '24), make it possible to correlate the development of the pronephros as here described with other growth processes in the embryo and with the development of the behavior of the embryos in a way that deserves notice.

THE NON-MOTILE STAGE

There are two nephrostomes in the non-motile stage, but the tubules and pronephric duct are solid cords of cells without a lumen excepting occasional clefts between the cells in the center of the cord. Pigment granules are grouped densely in the central ends of the cells. The yolk is distributed evenly throughout the cells. The cells of the nephrostome bordering on the coelom have fine ciliary processes.

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[1] For financial assistance in the prosecution of these studies the authors are indebted to the Research Committee of the Graduate School of the University of Kansas.

[2] As described by the senior author, Amblystoma embryos pass through the following definite stages in the development of behavior: the non-motile stage, characterized by movement in response to mechanical or electrical, but not to tactile stimuli; the early flexure stage, characterized by the earliest response to tactile stimuli; the coil stage, characterized by the tetanic contraction of the muscles of the entire trunk on one side only; the 'S' reaction, characterized by the earliest sinuous flexure, and the early swimming stage, which is the 'S' reaction performed in sequence and with sufficient power to produce locomotion.

THE EARLY FLEXURE STAGE

In the early flexure stage cilia are well developed on the nephrostome cells bordering the coelom and are beginning to appear on cells deeper in the nephrostome. The cilia are directed into the coelom, without any suggestion of their beating into the nephrostome.

The tubule at the level of the second nephrostome is slightly coiled and has a small lumen. The pronephric duct has a continuous lumen which opens into the cloaca.

THE COIL STAGE

In the coil stage the cilia in the nephrostome can be seen to arise from a definite cuticular border of the cells. They are now directed into the nephrostome toward the lumen of the tubule. The tubules are much more coiled than in the earlier stage, apparently having increased greatly in length. The lumen of the pronephric duct has between three and four times the diameter that it has in the early flexure stage. Blood corpuscles are now very numerous in the sinusoids around the tubules. The glomeruli have become well developed. The yolk has mostly disappeared from the part of the tubule cells next to the lumen, while the deeper parts are still well filled with yolk.

THE EARLY SWIMMING STAGE

The pronephros has in the early swimming stage become an intricate skein of coiled tubules. The coiling occurs especially between the levels of the second and third nephrostomes. The cilia are directed into the nephrostome. The cells of the nephrostome and tubules are smaller than in the coil stage, and have less yolk, the yolk globules being smaller than in the earlier stage and crowded into the base of the cells. The glomerulus has become a large sacculated structure that protrudes into the coelom. The diameter of the pronephric duct has not increased perceptibly since the coil stage.

CONCLUSION CONCERNING FUNCTION

Although the excretory duct of the glands has a lumen emptying into the cloaca at the early flexure stage and the renal glomeruli, according to Moore (loc. cit.), are then established upon the aorta the direction of the cilia of the nephrostomes, pointing into the coelom instead of into the tubules, indicates that no current can yet have been directed into the tubules. In the coil stage, however, the pointing of cilia into the tubules, the increase of the diameter of the lumen of tubules and ducts to virtually full size, and the reduction of yolk in the cells, particularly about the lumen, convince the writer that the pronephros has now acquired its definitive function. This is further supported by the observation by Moore (loc. cit.) that it is in the coil stage that the vascular circuit is first found to be complete.

DISCUSSION

Physiological correlation

While no effort has been made to demonstrate chemically the time when the characteristic secretions are first formed in the kidney, according to the observations of the junior author the pronephros first acquires the structure of a functional organ in the coil stage. The significance of this stage of development, it should be emphasized, is that the muscles of the trunk have attained to full power of contraction. The contractions in this stage are tetanic and the body is bent into a tightly coiled condition. The muscles now have apparently full strength and the animal would be capable of locomotion if the nervous coordinating mechanism were sufficiently developed. That the kidney should give the first evidence of beginning its function at the time when the vascular circuit is first completed (Moore, loc. cit.) is also a noteworthy fact. The elaboration of muscular energy in such an intensive manner must give rise to waste products which require elimination through the kidney. And while, according to Moore (loc. cit.), the muscles are not at this stage penetrated by blood vessels, the most highly differentiated muscle cells are in the ventral portion of the myotome (Faris, '24) in close proximity to the posterior cardinal veins (Coghill and Moore, '24). It is probable that this degree of vascularization of the tissues around the muscles is sufficient to remove the excretory products from the functioning muscle cells, inasmuch as contraction at this time is intermittent with relatively long periods of rest. It would appear to be, therefore, in part an adaptation to muscular function that the pronephros reaches the functional stage of development when it does.

The position of the pronephros

One of the very striking and mysterious incidents in the development of vertebrates is the succession of functional kidneys, which make their appearance first in the rostral part of the trunk and become successively more caudal in position. While it is not the thought of the writers to propose an explanation of these phenomena, it seems worth while to call attention to certain correlations that are obvious in the development of Amblystoma as observed by the senior author and his students, particularly Faris ('24), Moore (loc. cit.), and Soper. The senior author (Coghill, '24 b) has shown that up to and including the early swimming stage there is relatively very little differentiation in the central nervous system caudally of the ninth myotome level, whereas the differentiation in the medulla oblongata up to and including the coil stage is very great, and from the coil to the early swimming stage the differentiation rate in the cerebrum is relatively prodigious.

As correlated with these facts, Moore (loc. cit.) shows that there is precocious development of the anterior cardinal veins alongside of the medulla oblongata rostrally, that at the early flexure stage this vein connects with the posterior cardinal vein and ductus Cuvier, and that at the coil stage the vascular circuit is completed. Moore's observations show also that it is in the early swimming stage, when, according to the senior author's observations (Coghill, '16), the olfactory and optic nerves establish connection with the brain and when there is exceptional acceleration in differentiation in the cerebrum, that the vascular system invades the most rostral territory of the central nervous system. There can be no doubt but that the vascular functions in these early periods concern chiefly the rostral parts of the central nervous system, i.e., the several rostral segments of the spinal cord and the brain. Furthermore, according to Moore (loc. cit.), the function of the vascular system must be chiefly excretory during this period of development. Again, Faris ('24) has shown that processes of differentiation produce waste substances which are, in part at least, stored in the cells temporarily in an inert form. While it has not been shown just when and how the substances that are so stored are finally eliminated, it would appear that in the early period of development of vertebrates the paramount demand for renal function is in the head regions on account of the precocious differentiation of nervous tissue in relatively large masses in that region. It is possible, therefore, that adaptation to this condition has been a factor in establishing the relatively rostral position of the pronephros.

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THE GROWTH OF THE HEAD IN THYROIDECTOMIZED SHEEP

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EIGHT FIGURES

When young lambs are thyroidectomized, growth is soon retarded and the effect is the greater the earlier the operation is performed. These animals seldom live more than two years, but in three thyroidectomized sheep which survived longer the development of an undershot jaw was observed for the first time. This is shown in figures 1 and 2. In this animal, three years six months of age, the snout is wrinkled and depressed and the lower jaw protrudes to such an extent that the lower teeth are exposed. Figures 3, 4, and 5 show this cretin sheep and its twin control at various ages.

They were born in May, 1921, and one of them was thyroidectomized at four weeks of age. The photograph taken in October of the same year (fig. 3) shows no obvious peculiarity in the conformation of the cretin sheep's head, although it is already dwarfed in stature. In April, 1923 (fig. 4), when the thyroidectomized animal was almost two years of age, the prominence of the lower jaw is apparent, and by October, 1923 (fig. 5), the lower teeth are exposed.

Another cretin sheep of the same age as the one just described has likewise developed an undershot jaw. Figure 6 shows this animal at three years six months of age. The

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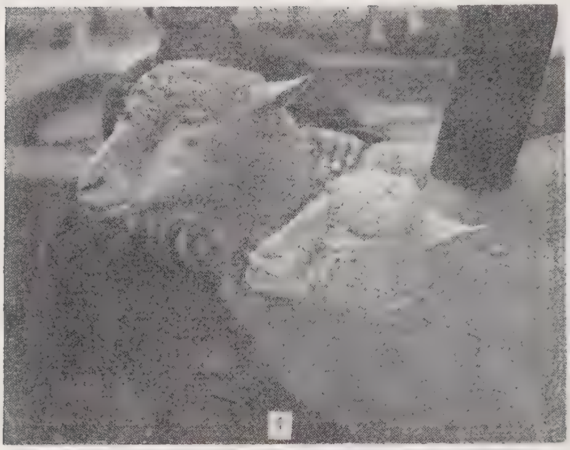
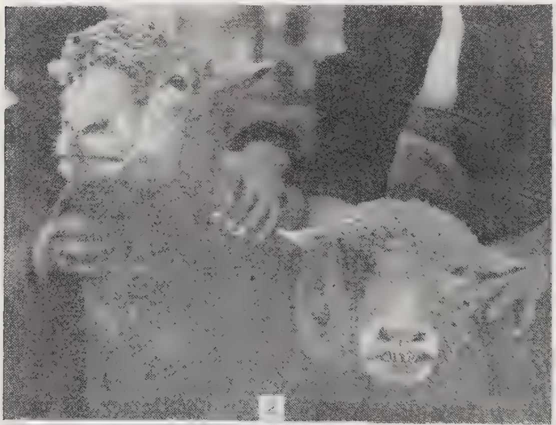
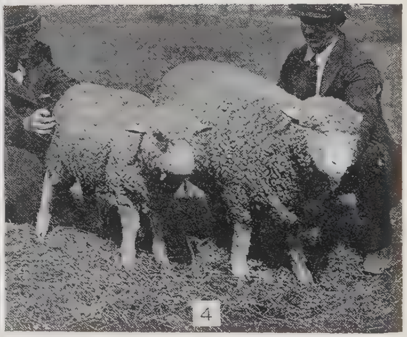
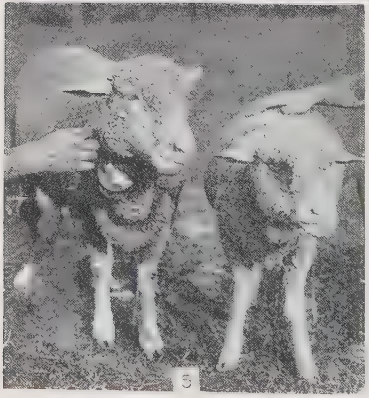








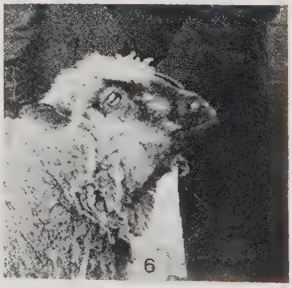
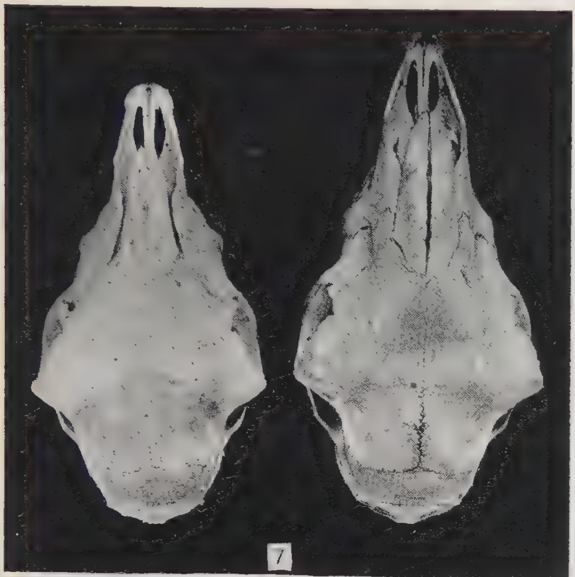


Fig. 1 Thyroidectomized sheep and her twin control. Note the undershot jaw of the cretin.

Fig. 2 Same twins as in figure 1.



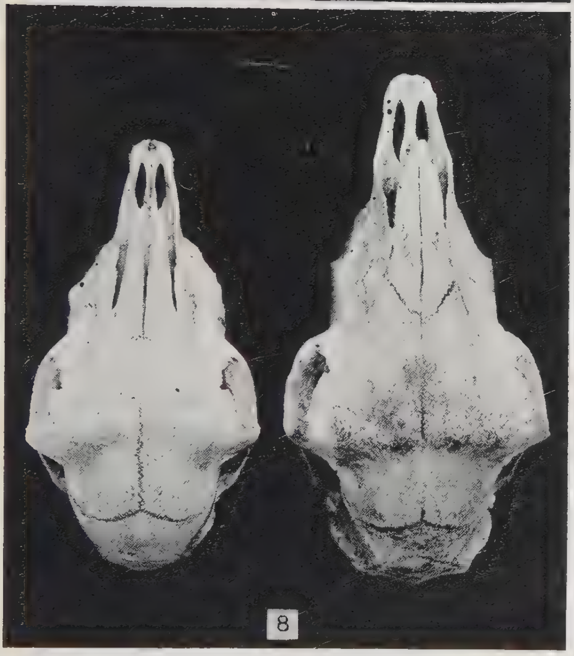


Fig. 7 Skulls of thyroidectomized sheep (Dop) and her normal twin sister. The cretin skull is at the left.

Fig. 8 Skulls of thyroidectomized sheep (4op) and her normal twin sister. The cretin skull is at the left.

same feature is developed to about the same degree in a thyroidectomized wether two years six months old. The thyroids were removed from this animal fourteen weeks after birth. However, another cretin, born at the same time and thyroidectomized at the same age, does not exhibit a bulldog jaw, although it is much more stunted than the wether.

When one of the cretins of Dr. Sutherland Simpson's experimental flock dies, its normal twin is killed and the tissues of both are fixed and embedded for microscopical examination. Formerly the skeletons were discarded, but recently it was decided to preserve them for detailed study. We have so far had the opportunity to examine the skulls of but two pairs. One twin of the first pair (Dop) was thyroidectomized when 105 days old and died nine and one-half months later. At death the body weight of the cretin was 70 per cent that of its normal sister. Another twin lamb (4op) was thyroidectomized at four weeks of age and survived two years. At death its normal twin was killed and the body weight of the cretin was 45 per cent that of the control. The skulls of these two pairs appear in figures 7 and 8.

The development of a detailed system of measurements has been postponed until more skeletons were available. However, an examination of the data in table 1 reveals the significance of the apparent overdevelopment of the mandible in the cretins pictured above.

Although neither of the thyroidectomized animals whose skulls are shown in figures 7 and 8 exhibited a protruding lower jaw, a conformation of the skull is seen, more pronounced in 4op than in Dop, which if further developed would eventually have resulted in the protrusion of the mandible. Both dwarfed skulls are foreshortened and disproportionately wide. This is shown in the length-breadth index which is greater for each cretin skull than for the corresponding control. This rounding of the skull is well known in the human cretin and has been observed by Biedl(1), von Eiselsberg(2), and others in various mammals following thyroidectomy. It should also be noticed (table 1) that the nasal and frontal bones are more retarded in growth than the parietal and occipital.

Defective dentition is observed in both cretin skulls. It is more pronounced in the older, 4op, where none of the premolars have completely erupted and the third molars are just visible. Another interesting feature of the cretin skulls is that ankylosis of the basisphenoid and basioccipital is incomplete.

TABLE 1

Measurements of normal and cretin sheeps' skulls. Measurements 4 to 9 were between points of the sliding compasses in the sagittal plane

	D CONTROL	DOP.	4 CONTROL	4OP.
1. Greatest width: tip of premaxillaries to occipital protuberance	22.3 cm.	19.9 cm.	22.7 cm.	19.1 cm.
2. Greatest width: between orbital processes of the frontals	11.1 cm.	10.3 cm.	11.6 cm.	10.2 cm.
3. Index: $\frac{\text{width} \times 100}{\text{length}}$	49.8	51.8	51.1	53.4
4. Nasal chord: tip of nasals to nasofrontal suture	8.2 cm.	6.7 cm.	8.5 cm.	6.1 cm.
5. Frontal chord: nasofrontal suture to frontoparietal suture	7.9 cm.	7.6 cm.	8.3 cm.	7.1 cm.
6. Parietal chord: frontoparietal suture to parieto-occipital suture	3.1 cm.	2.9 cm.	2.1 cm.	3.4 cm.
7. Occipital chord: parieto-occipital suture to opisthion	3.6 cm.	3.4 cm.	4.6 cm.	3.2 cm.
8. Basioccipital length	2.6 cm.	2.3 cm.	2.5 cm.	2.3 cm.
9. Basisphenoid length	2.7 cm.		2.7 cm.	2.3 cm.
10. Length of mandible	19.4 cm.	14.6 cm.	18.2 cm.	15 cm.

The hypophysis was removed in all four cases by trephining the basisphenoid, thus destroying the sella turcica, and unfortunately the weights of the glands were not recorded at the time. However, Simpson and Hunter(3) state: "Compared with normal animals of the same age there is evidence of some increase in the size of the pituitary in the thyroidectomized lambs and sheep, but this is not so great as has been reported by many observers in other experimental animals,—notably the rabbit,—nor in some cases of myxoedema and cretinism in the human subject. In the case of the lambs this increase amounted to about 15%, and in the adult sheep to about 20%, in from five to six months."

SUMMARY

In thyroidectomized sheep which have survived for more than two years an undershot jaw has been observed. In younger cretins a conformation of the skull is present which, further developed, would result in this protrusion of the mandible. The skull is foreshortened and disproportionately wide. Delayed eruption of the teeth is observed.

Unlike the skulls of the normal animals, ankylosis between the basisphenoid and basioccipital is incomplete in both cretin skulls.

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3 SIMPSON AND HUNTER 1911 Quart. Jour. Exper. Physiol., vol. 4, p. 271.

THE EFFECT OF NATURAL FOODS ON THE DISTENTION OF THE GALL BLADDER, WITH A NOTE ON THE CHANGE IN PATTERN OF THE MUCOSA AS IT PASSES FROM DISTENTION TO COLLAPSE

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ONE TEXT FIGURE AND THREE PLATES

In the course of dissecting a large number of cats, examined immediately after death for the presence of pancreatic bladders, the writer's attention was attracted by the not infrequent occurrence of collapsed gall bladders. Since all gradations between these and fully distended vesicles were also present, it was apparent that different physiological states of this organ, as interrupted by death, were here being displayed. This observation suggested the possibility of inducing a collapse of the gall bladder by feeding cats with appropriate food. Assuming that the most effective substances would be those which are digested primarily through the agency of the bile, the first diet selected was a combination of egg yolk and heavy cream—substances known to produce striking visual evidence of the absorption of fat by the lacteals. Cats fed upon this diet at once exhibited a functional periodicity of the gall bladder in relation to meals, each cycle consisting of three main phases: a period of slow emptying, a collapsed or resting period marked by a rearrangement of the mucosa, and a period of slow filling and bile concentration. Pure protein, on the other hand (administered in the form of egg white), and carbohydrate (in the form of rice), failed to cause any marked diminution in the size of the gall bladder. But the striking results obtained with yolk and cream suggested that through appropriate diet the human gall bladder might be thoroughly emptied in a way valuable for clinical purposes, and accordingly these results were reported at the Boston meeting of the American Association for the Advancement of Science.[1] Before publishing the details of this work, however, it was intended to apply these observations to various problems that could be solved only by recourse to operative procedures. While awaiting an opportunity to do this work, the situation was suddenly changed by the publication of the brilliant experiments of Graham and Cole,[2] establishing a method for rendering the gall bladder opaque to Roentgen rays. For the first time direct observation of the human gall bladder in its relation to the food cycle was made available. It seemed desirable, therefore, to publish the details of this work at once, in the hope that it might be of some use to other workers in this field.

Through the generous cooperation of Doctor Sosman (Roentgenologist at the Peter Bent Brigham Hospital) and Doctor Whitaker (Research Fellow in charge of the Laboratory of Surgical Research) I have recently been given the opportunity of applying to the human body certain diets that have proved most effective with cats. Previous to this, without at first knowing of the work on the cat, these investigators had noted that cholecystograms of the human gall bladder, taken shortly after meals, showed marked reduction in size, butter being an especially effective reducing agent.[3] But no such effect was produced as was at once obtained when the writer was x-rayed after eating the yolk of four raw eggs and a half pint of cream.[4] In one hour and forty minutes after the beginning of this meal cholecystograms showed that the gall bladder had shrunken from the fully distended condition imposed by going without food for eighteen hours (fig. 2) to the phase of collapse exhibited in figure 3. (Note that in the fasting condition the gall bladder extended from the second to the fourth lumbar process, whereas in figure 3, taken with precautions to obtain the same orientation though the chance for error is considerable, its length was reduced to the distance between the second and third transverse processes.) In another hour and twenty minutes (three hours after eating) no shadow of the gall bladder could be obtained at all, the assumption being that all iodinized bile had been expelled from the gall bladder with the total collapse of its walls.

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[1] The gall bladder in the cat—its development, its functional periodicity, and its anatomical variation as recorded in 2500 specimens. (Abstracted in Anat. Rec., vol. 24, Jan., 1923, p. 388.)

[2] Roentgenologic examination of the gall bladder. J. A. M. A., 1924, vol. 82, pp. 613–614.

[3] The clinical use of sodium tetraiodophenolphthalein in cholecystography. Surg. Gyn. and Obs., May, 1925, p. 649.

[4] The technique employed in rendering the gall bladder opaque to the x-ray was that perfected by Doctor Whitaker and his associates (The oral administra-

In the remaining three figures of plate 1, from the same individual, may be seen the effect upon the gall bladder of eating 9 ounces of rump steak from which all visible trace of fat had been removed. In figure 4, taken the same length of time after eating as figure 3, the gall bladder is nearly twice as long and somewhat wider, although it is markedly reduced from the fasting condition shown in figure 2. In figure 5, taken three hours after the meal of steak, the gall bladder is still contracting, but it has presumably reached its lowest point, for in two hours more (fig. 6, five hours after eating) it is becoming filled again, being well on its way toward distention.

The writer has chosen these two diets to demonstrate two things: 1) the existence in man of a functional cycle of the gall bladder comparable to that in the cat and, 2) the inferior effect of a meat diet on this cycle as compared with a diet rich in fats and lipoid substances. The significance of the

tion of sodium tetraiodophenolphthalein for cholecystography, Surg. Gyn. and Obs., June, 1925). In this case twelve pellets (5 grains each) of the sodium salt were taken after a light supper, the pills having been coated with stearic acid to prevent irritation of the gastric mucosa with consequent vomiting and nausea. Eighteen hours later, sufficient amount of the salt had been excreted with the bile to give a satisfactory shadow of the distended gall bladder. Appropriate food was then eaten and the gall bladder photographed at desired intervals.

first lies in the light it throws on the function of the gall bladder and on the possibility it offers for emptying the gall bladder in clinical cases; the importance of the second fact rests upon the circumstance that those who have investigated the factors controlling the secretion of bile have emphasized the value of a meat diet in producing the maximum flow of bile through a fistula.[5] It is possible to explain this apparent discrepancy—namely, that meat diet causes the greatest production of bile and a fat diet, the maximum emptying of the gall bladder—assuming that both statements are quite true by postulating that two mechanisms may be involved, one which regulates the emptying of the gall bladder and is best initiated by a diet of fat and lipoids, and another which regulates the secretory activity of the liver and is best initiated by a diet of meat. It is the former of these conditions only which I have studied and apparently established; but, as will be pointed out later, these discrepancies can be reconciled in another way if we assume that no lipoids were present in the fats used by these investigators.

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[5] Starling ('20, p. 763). ''The effect of various diets on the secretion of bile has been studied by Barbera. He finds that, whereas the secretion of bile is greatest on a meat diet, it is somewhat less on a diet of fat, and is insignificant on a purely carbohydrate diet. That is to say, the secretion of bile is greatest on those diets the digestion of which is attended by the passage of a large amount of acid chyme or of oil into the duodenum.'' Also Whipple (Physiol. Review, July, 1922, p. 449) makes the following statement regarding chologogues: ''We may note the few points about which all workers are in accord. Bile salts by mouth or intravenously will cause a distinct rise in the volume of bile secreted by a temporary or permanent fistula. Likewise, a meat diet favors an abundant flow of bile in a bile fistula dog but a diet rich in carbohydrates, especially sugar, will decrease the bile flow under similar conditions . . . . This shows the importance of diet as influencing the bile flow.'' In discussing other drugs that have been proposed as chologogues, he continues: ''If chologogue action is present it is slight, or delayed or transient and is usually less than can be demonstrated as due to food factors (meat, for example).'' A similar opinion is expressed by McMaster, Broun and Rous (Studies on Total Bile, Journ. Exp. Med., 1923, vol. 37, p. 417). Pawlow, on the other hand, who was presumably the first to study intensively the effect of diet on the secretion of bile, writes as follows: ''One cannot resist the idea that the bile has quite as important a part to play in the chemical elaboration of food as any of the other digestive juices. Hence we proceeded further in our investigations, and gave the ingredients of the food separately to the dogs to eat, or brought them directly into the stomach. It resulted that neither water, acids, raw albumen nor boiled starch paste, whether in solid pieces or as a thin fluid, caused a flow of bile. Fat, on the other hand, as well as the extractives of meat, and the products of digestion of egg albumen, set up a free discharge of the fluid. The bile, therefore, resembles other digestive juices in that it possesses its own particular combination of excitants, which have the effect of causing it to flow into the intestine.'' (The Work of the Digestive Glands. London, 1902, p. 156.)

A third problem upon which this work has a direct bearing, and which will be more carefully discussed in what follows, is the question of how the collapse of the gall bladder should be interpreted. Does this organ expel its contents through the cystic duct or, as some recent authorities contend, is it emptied entirely by absorption through the mucosa? Before entering upon this problem, which is not as simple as it might seem, it is desirable to examine closely the cycle of changes in the cat, since observation of experimental animals offers advantages which cannot be duplicated by cholecystography of the human body.

In table 1 of this paper is presented a résumé of the original results obtained by feeding a mixed meal of egg yolk and heavy cream to thirty-seven adult cats. As recorded in the fifth column, the cats were examined under anesthesia at graded intervals after feeding. The horizontal lines subdivide the cycle into its three phases. Thus in about an hour and three-quarters after meals the gall bladder has passed from a fully distended to a nearly collapsed state (requiring for this procedure the same time as was subsequently established for the human gall bladder). For nearly five hours thereafter it remains more or less collapsed, reaching its smallest diameter six and one-half hours after feeding. Then it commences to fill, becoming fully distended about ten hours after the meal.

The first of these phases—the period of slow emptying—is characterized by the peculiar behavior of the wall of the gall bladder. As the organ diminishes in size, it does not remain circular in cross-section like the urinary bladder, which presses closely against the contained fluid, but becomes flabby as if it were being sucked down by the withdrawal of bile. This becomes especially noticeable at the end of this period; for if the liver be then tipped back, so that the few drops of bile which remain in the gall bladder run into the neck, the fundus flattens out. Elementary as this observation is, it would seem to indicate that the bile is not expelled by any simple or ordinary contraction of its intrinsic muscle tunic.[6]

A second characteristic of this period is the inspissated nature of the bile within the gall bladder which is always dark green and somewhat viscid. By the time the bladder has collapsed only a ropy residue remains, indicating that the process of concentration continues during the emptying of the biliary reservoir.

The second phase, the period of maintained collapse beginning one hour and three-quarters after meals, is the longest of the three, lasting approximately five hours. I have defined it also as a resting period, since the gall bladder during this time is virtually free from distention. Of the twenty-three animals examined in this interval of five hours, all but five possessed collapsed or nearly collapsed gall bladders. But following the third hour after meals the appearance of a few drops of yellow bile in the gall bladders of many of the specimens makes it certain that this organ is not en-

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[6] In the clinical literature this finds expression in William Mayo's familiar saying that "no one has ever seen the gall bladder contract . . . . to force the bile out at proper intervals (J. A. M. A., vol. 56, 1911, p. 1021. "Innocent gall stones a myth"). In the latest summary of the functions of the gall bladder (Physiol. Review, April, 1924, p. 262, etc.), F. C. Mann writes the following interesting résumé: "The smooth muscle fibres in the wall of the gall bladder form an anatomic basis for this action (contraction). These fibres seem to be present in all specimens examined, although data concerning this are not complete. In any event the muscle coat must be extraordinarily thin in some specimens. The action of this muscle is a crucial consideration with regard to the function of the gall bladder: does the muscle empty the gall bladder or simply aid in preventing distention? . . . . Measurements have never been made of the force of contraction of the gall bladder that are totally free from objections. There seems to be no doubt that the gall bladder has a contractile mechanism which exerts pressure on its contents, but the contractile power of its own intrinsic muscles does not seem to be great, and in all probability it cannot exert a pressure greater than the secretory pressure of the liver." (According to Whipple, loc. cit., this in cats and dogs is between 210 and 375 mm. of bile.)

TABLE 1

Egg yolk and heavy cream

CASE NO.	HOURS OF FASTING	YOLK	CREAM	HOURS POSTCIBAL	LACTEALS	GALL BLADDER AND BILE
2606	17	3	40 cc.	0.33		Distended, thin-wall; dark green, viscid
2602	17	3	30 cc.	0.66		Moderately full, loose walled
2050	17	2½	120 cc.	0.8	Cloudy	Moderately full, loose walled; dark green, viscid
2601	48	3	30 cc.	1	Cloudy	Moderately full, loose walled; dark green, viscid
2598	17	3	90 cc.	1	Cloudy	Moderately full
2599	24	2½	30 cc.	1.25	White	Moderately full; dark green, viscid
2051	16	1	Little	1.30	Cloudy (?)	Moderately full
2595	24	2	45 cc.	1.75	Cloudy	Moderately full; dark green, viscid
2594	17	3	30 cc.	1.75	Very white	Nearly collapsed; small amount dark green
2417	17	3	48 cc.	2.25	Very white	Nearly collapsed; small amount dark green
2412	45	2½	15 cc.	3	Very white	Collapsed; dark green, mucous residue
2052	15	4	Little	3	Very white	Collapsed; flat, thick walls; ropy residue
2225	48	2	40 cc.	3	Very white	Collapsed; brownish green, mucous residue
2579	22	2	Little	3	Very white	Collapsed (round); few drops yellow green
2537	24	2	Little	3.2	Very white	Nearly half full; golden green
2587	24	3	45 cc.	3.3	Very white	Half full; green, tinge of yellow
2536	17	4	Little	3.5	Very white	Nearly half full; tinge of yellow
2593	20	1	60 cc.	3.75	Very white	Quarter full, round; clear yellow
2588	24	1	45 cc.	3.75	Very white	Nearly collapsed; small amount dark green
2418	17	1	Little	3.75	Very white	Collapsed, round; small amount dark green
2413	45	2½	15 cc.	4	Very white	Collapsed; drop
2623	17	4	Little	4.33	Very white	Nearly collapsed; 2 drops yellow green
2533	17	2½	Little	4.5	Very white	Nearly collapsed; small amount
2580	40	4	Much	4.75	Very white	Nearly collapsed, flat; few drops yellow
2226	48	2	40 cc.	5	Very white	Collapsed, pinkish white; ropy residue
2495	48	3	60 cc.	5.75	Very white	Collapsed, flat, pinkish white; dark green
2619	17	2½	30 cc.	6	Very white	Nearly collapsed, flat; few drops, golden yellow
2414	45	3	48 cc.	6	Very white	Half full; golden green, somewhat viscid
2496	48	3	48 cc.	6.33	Very white	Collapsed; ropy residue
2497	48	3	48 cc.	6.5	Very white	Maximum collapse; thick and round; thin film of residue, no fluid content
2578	20	3	Little	6.75	Very white	Maximum collapse; thick and round; drop of yellow in neck
2521	17	3	48 cc.	7	Very white	Half full; golden yellow
2616	17	2	30 cc.	7.5	Very white	Half full; golden yellow
2520	17	3	120 cc.	7.66	Very white	Quarter full; dark green, viscid
2227	48	3½	80 cc.	8	White	Half full and round; two lobes yellow, one lobe golden green
2498	48	2	48 cc.	9	Very white	Half full and round; dark green
2577	20	3	Little	10	Very white	Fully distended; yellow
2576	20	0		17	Collapsed & colorless	Fully distended; dark green, viscid
2224	48	0		48	Collapsed & colorless	Fully distended; dark green, viscid

tirely quiescent throughout the five-hour period. Through the intermittent action of the sphincter papillae it undoubtedly receives small accessions of new bile from the hepatic ducts. More detailed analysis of this condition must necessarily await experimental procedures. But attention was attracted to the fact that four of the five gall bladders which were not collapsed during this five-hour period were half filled with yellow-green bile between the third and fourth hours after meals; according to the literature this is the period which just precedes maximum bile secretion following a meal of fat, as determined by experiments employing fistulas of the common duct.[7] One would be inclined to consider these four cases exceptional—as specimens that had lagged behind the others in emptying or that had begun to fill sooner—were it not for the fact that the same phenomenon is also suggested by the tables recording the effect of a meat diet and of a yolk diet.

It therefore happens, on account of this inflow of new bile and because of the concentrating activity of the mucosa, that the gall bladder, although collapsed, is never entirely empty. Either it contains a drop or two of yellow fluid or a tarry residue of inspissated bile left clinging to the wall. But it is nearly if not quite empty throughout most of this five-hour period. The maximum reduction in size seems to be reached about six and one-half hours after meals. Two such cases (and many others approaching this condition) were contracted into hard thick-walled tubular organs, with a rearranged mucosal pattern which will be discussed at the end of the paper (fig. 8). This shows that the thin musculature of the gall bladder, if given sufficient time, eventually takes up the slack in the wall and produces a cylindrical organ.[8]

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[7] According to Ellenberger and Schleunert (Lehrb. d. vergl. Physiol. d. Haussäugetiere, 1910, p. 338), "the maximum secretion begins with a meat diet in the fourth, with a fat diet in the fifth, and with a carbohydrate diet in the third hour, then slowly diminishes. With a fat diet the secretion of bile was meagre and first began to increase an hour after eating and reaches its maximum after three hours. Still more meagre is the secretion following carbohydrates, which begins almost immediately after eating and often even after six hours. With egg white and meat diet the bile secretion was most marked and active after eight hours; the increase setting in one-half hour after meals and reaching its maximum after four hours." It is possible, however, that a fat diet containing lipoids might be found to modify the time of maximum secretion very considerably. This should be investigated before any conclusions are drawn about the significance of this period of semidilation in the midst of this resting period of the gall bladder.

The third period in this functional cycle is initiated about six and a half hours after feeding by a rather sudden inflow of hepatic bile which partially fills the gall bladder. By the tenth hour the vesicle has become fully distended with yellow bile, which thereafter undergoes concentration into a dark green sirupy fluid.

A review of the conditions found in these thirty-seven animals may be summarized as follows. Beginning at the irregular level maintained by a fasting animal, the distended gall bladder nearly collapses within an hour and three-quarters after a meal of yolk and cream; during the next five hours it remains virtually collapsed, but receives, intermittently, small increments of yellow bile which, except for the first one, rarely amounts to more than a few drops. During this period of collapse its walls are generally flabby and flattened, but toward the end of the period the slack in the wall is usually taken up so that it becomes a fully contracted, thick-walled cylindrical organ. Immediately afterward the inflow of new bile, more rapid at first than later, restores the distended fasting condition.

Following the demonstration of a functional cycle induced by a mixture of yolk and cream, other natural foods were tried. The first of these was horse meat, a food commonly given to the denizens of the animal farm (A, table 2). But this proved not to be so effective in reducing the gall bladder as yolk and cream. The number of animals used was smaller, but they agreed in showing a cycle similar to that obtained

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[8] The color of the gall bladder varies with its content: if the contained bile is dark green, the wall is bluish green; if yellow, that color is visible through the transparent walls; if only a ropy residue remains, the gall bladder resembles a collapsed stomach—the gray-blue sheen of the serosa being tinted with red on account of the greater relative volume of blood vessels.

TABLE 2

A. Horse meat

CASE NO.	HOURS OF FASTING	FOOD	HOURS POST-CIBAL	LACTEALS	GALL BLADDER AND BILE
2403	17	$\frac{1}{4}$ lb. lean	3	Cloudy	Moderately full; dark green, viscid
2632	17	$\frac{1}{4}$ lb. rare	3.25	Cloudy	Half full; dark green, viscid
2404	17	$\frac{1}{5}$ lb. lean	3.5	Cloudy	Fully collapsed; green ropy residue
2411	45	$\frac{1}{4}$ lb. rare	3.75	Slightly cloudy	Nearly full; dark green, viscid
2419	17	Small amount, fatty	3.75	Cloudy	Nearly full; dark green, viscid
2581	17	$\frac{3}{16}$ lb. lean	4	Slightly cloudy	Half full; yellow green
2494	48	$\frac{1}{4}$ lb.	4.25	Cloudy	Nearly collapsed; greenish brown, ropy
2228	48	$\frac{1}{4}$ lb. lean	5	Slightly cloudy	Nearly collapsed, flat; dark green ropy residue
2635	17	$\frac{1}{4}$ lb. rare	5.5	Cloudy	Half full; dark green, viscid
2405	17	$\frac{1}{7}$ lb. rare	6	Cloudy	Moderately full; dark green, fluid
2229	48	$\frac{1}{4}$ lb. rare	8	Cloudy white	Nearly full; dark green, fluid
		B. Yolk and meat			
2408	17	1 yolk, $\frac{1}{8}$ lb. meat	2.75	Very white	Collapsed; ropy greenish brown residue
2631	17	1.5 yolks, $\frac{3}{16}$ rare meat	3.25	Very white	Collapsed, flat; 2 drops, golden yellow
2418	17	1 yolk, fatty meat	3.75	Very white	Collapsed, round, thick walled; green fluid
2409	17	2 yolks, $\frac{3}{8}$ lb. meat	4	Very white	Nearly collapsed; small amount dark green, ropy
2634	17	4 yolks, $\frac{3}{16}$ lb. rare	6.5	Very white	Nearly collapsed, oval; 3 drops thick golden yellow

C. Yolk

2630	17	2.5 yolks	2.66	White	Collapsed, flat; 2 drops dark green, ropy
2575	17	2.5 yolks	3	Very white	Nearly collapsed, flat; dark green?
2574	17	4 yolks	4.5	Very white	¾ collapsed, flat; dark green?
2582	17	4 yolks	6.5	Very white	Nearly collapsed; dark green, ropy
2633	17	3 yolks	6.5	Very white	Collapsed, flat; 1 drop, golden yellow, viscid
2590	36	4 yolks	7.5	Very white	Nearly collapsed; yellow green, ropy

D. Cream

2584	17	75 cc. cream	2.66	Very white	Nearly full; dark green, viscid
2583	17	78 cc. cream	4	Cloudy	Full; dark green
2586	17	81 cc. cream	5	Cloudy	Half full; dark green, ropy
2589	36	120 cc. cream	5.5	Cloudy	Half full; dark green, ropy

E. Egg-white

2416	17	4 cooked in Liebig's	2.5	Clear	Nearly full, walls loose; dark green, ropy
2585	17	4 cooked with sprinkling meat juice	3.5	Clear	Full; dark green, ropy
2410	45	6 cooked in Liebig's	4	Clear	Moderately full; dark green, viscid
2401	17	2 uncooked	4.25	Cloudy	Nearly full; golden yellow
2402	17	3 uncooked	5.25	Clear	Full; dark green, fluid

F. Rice (sprinkled with Liebig's extract)

2406	17	2½ heaping tablespoonfuls	2.5	Clear	Moderately full; dark green, viscid
2407	17	3 heaping tablespoonfuls	4	Clear	Moderately full; dark green, viscid

from a yolk-and-cream diet, only not so pronounced—the gall bladder collapsing more slowly, not staying down so long, and filling sooner. Yolk and meat (B, table 2), however, were nearly as effective as yolk and cream, and the same was true of yolk alone (C, table 2). Cream (D, table 2), on the contrary, and fish produced only partial collapse of the gall bladder, and protein in the form of egg white (E, table 2) and carbohydrate in the form of rice (F, table 2) were almost ineffectual.

The immediate result of this comparative study, then, was to fix the attention on yolk as the most significant food, the next step being to segregate and separately administer the substances of which it is composed. The albumen and mineral salts were discarded, since they also occurred in egg-white, which was proved to be inert in its effect on the gall bladder. That left only three constituents which seemed promising, namely, the fats (olein, palmitin, and stearin, which constitute approximately 22 per cent of the yolk), and the lipoids (lecithin, approximately 10 per cent, and cholesterol, less than 2 per cent). With the advice of Prof. C. H. Fiske, who has helped me freely in regard to the chemical aspects of my work, three substances were chosen which contained the above ingredients, namely, lard (containing the three fats), lecithin (90 per cent, prepared from eggs by Merck), and lanolin (wool-fat, containing large amounts of cholesterol). Each of these substances was mixed with cream and fed by stomach pump to fasting cats. They were also administered without the addition of cream, with the exception of lanolin, which could not be retained alone in the stomach. Results of these tests and their controls (yolk and cream) are given in table on following page.

A comparison of the effects of these three substances at once points to lecithin as the most effective of the three components of the yolk, since cats fed with this substance paralleled the behavior of the controls fed with yolk and cream.[9]

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[9] The failure of the gall bladder in both controls and lecithin-fed animals to show a complete collapse at 2.75 hours is presumably due to the effect of ether and the irritation of the stomach pump; four hours later, this initial handicap had been almost overcome.

Lard, containing the fats which occur also in cream, showed about the same effect as cream alone (table 2, D) causing a delayed semicollapse of the gall bladder. This explains the efficacy of a mixture of yolk and cream, the yolk operating to produce extensive collapse within the first two hours after meals, the cream reinforcing the continuing action of the yolk several hours later, the two together producing a maximum collapse at about six and one-half hours. It also explains why fats (not mixed with lipoids) have failed to attract the same attention as meat, in the effect on bile secretion. The third substance, lanolin, proved very hard to administer, causing salivation and nausea with subsequent vomiting. The one case in which it was retained indicates that it has little effect toward emptying of the gall bladder.

CASE NO.	FOOD	HOURS POST-CIBAL	LACTEALS	GALL BLADDER AND BILE
2626	4 yolks; 30 cc. cream	2.75	White	¾ collapsed; dark green, ropy
2620	5 gms. lecithin; 30 cc. cream	2.75	White	¾ collapsed; dark green, ropy
2621	Lard, size of egg; 30cc. cream	2.75	Cloudy	Nearly full; ropy
2625	3 yolks; 30 cc. cream	6.5	White	Collapsed; 1 drop, viscid, yellow
2624	Lecithin, size of yolk	6.5	White	Collapsed; 2 drops, viscid yellow
2622	Lecithin, size of yolk; 30 cc. cream	6.5	Very white	Collapsed; 2 drops, viscid yellow
2636	90 cc. lard	6	Cloudy	¾ collapsed; 7 drops, golden brown, viscid
2626	90 cc. lard; 60 cc. cream	6.5	Very white	¾ collapsed; 14 drops, dark green, viscid
2627	½ oz. lanoline; 45 cc. cream	6.5	White	Nearly full; dark green, viscid

A further analysis of lecithin, as pointed out by Dr. Fiske, should lead to the consideration of cholin, a product of the hydrolysis of lecithin. Already this substance has been considered in its relation to the gall bladder in a paper published some time ago by H. C. Wood, Jr.,[10] for which reference I am indebted to Dr. Reid Hunt. In experimenting on dogs with biliary fistulas, Wood and Asher found that intravenous injections of 5 per cent cholin caused a sudden but temporary rise in the bile secretion of fasting animals. They also noticed that the bile which flowed after cholin was introduced was darker in color than normal; this suggested the idea that the increase in rate of flow was due to a spasm of the gall bladder which forced out the concentrated bile contained in this organ. To test this they withdrew the bile, inserted an empty balloon into the gall bladder, and then inflated it with air. When there was no longer any bile or room for bile in the gall bladder, intravenous injection of cholin failed to increase the flow of bile in the common duct.

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[10] The pharmacological action of cholin. Phila. Monthly Med. Journ., July, 1899.

Though the above experimental procedure is not beyond criticism, this work of Wood's suggests that cholin may be the important factor in inducing a collapse of the gall bladder, and since, as a constituent of lecithin, it is abundant in the foods which I have found most effective for that purpose, his conclusion is confirmed to that extent.[11] But Wood, in referring to a spasm of the gall bladder adopts the idea that its musculature is sufficient to empty it. That was more clearly expressed by Foster,[12] who held that normally no bile is discharged into the intestine until the acid contents of the stomach are poured over the duodenal papilla. When that happens, he says, "the discharge is due to a contraction of the muscular walls of the gall bladder and ducts, accompanied by a relaxation of the sphincter of the orifice; both acts are probably of reflex nature, but the details of the mechanism have not been worked out." Since then the efficacy of the musculature has been doubted by clinicians and others, as we have already noted. Recently, two authors working independently from different viewpoints have seriously questioned the emptying of the gall bladder by way of the cystic duct—Sweet,[13] in this country, and Halpert,[14] in Prague (now at Johns Hopkins). The former bases his contentions on the mechanical hindrances to the outflow of bile and the well-known absorptive power of the wall of the gall bladder, so clearly demonstrated by Rous and McMasters.[15] Halpert finds in this conception the best explanation of biliary stasis, with the formation of calculi, which often occurs in the absence of any demonstrable occlusion of the cystic duct. If it could be proved that the gall bladder never emptied through the cystic duct, then anything which interferes with absorption would account for biliary stasis and its consequences. Halpert also stresses the mechanical hindrances to the outflow of bile:

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[11] Wood also refers to Brieger's discovery (in 1885) of cholin as one of the ptomains in putrefying meat. If cholin should prove to be the principal factor concerned in the collapse of the gall bladder following a diet of yolk, its presence, in lesser quantity, in meat as a split product of lecithin may explain the effect of meat in causing a partial collapse of the gall bladder.

[12] Text Book of Physiology, 5th ed., 1889, Pt. II, Bk. II, p. 434.

> The broad fusion of the body of the gall bladder to the liver, the almost parallel position of its axis to the long axis of the body, the fact that the fundus usually reaches lower down than the papilla of Vater, the complicated bends in the neck of the gall bladder, the arrangement of the Heisterian valves, as well as the striking narrowness and length of the cystic duct and its exit from the gall bladder by means of an eccentric small fine opening, the relations of the cystic duct to the ductus hepaticus and the manner of its union with the ductus choledochus—all these, together with the weakness and arrangement of the musculature of the gall bladder, almost completely exclude the possibility that the gall bladder can normally empty itself by its own power, through the cystic duct.

Finally, having demonstrated the passage of anisotropic lipoids through the mucous membrane of the gall bladder, Halpert concludes that all the bile within the gall bladder "is brought back through the lymph and blood streams into the domain of the body." The experimental proof for this conception, he admits, is still missing, yet, he says, "from my observations I venture to assume that the healthy gall bladder can resorb the healthy secretions of the liver in toto."

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[13] The gall bladder: its past, present, and future. Internat. Clinics, vol, 1, series 34, 1924.

[14] Neue Wege in der Gallenblasenforschung. Med. Klinik, Nr. 13 and 52, Jahrg. 1924.

[15] The concentrating activity of the gall bladder. Journ. Exp. Med., vol. 34, 1921.

These independent efforts of Sweet and Halpert to demonstrate that the bile leaves the gall bladder only by resorption, the statement of Foster that the muscle coat empties the gall bladder into the intestine after meals (virtually repeated more recently by Starling), and the comment of Mayo that no one has ever seen it happen are sufficient reasons for recording further details of the postprandial collapse. Either a very rapid absorption of bile through the wall of the gall bladder takes place after a meal of fat, the purpose of which would be obscure, or else bile is discharged through the cystic duct in some very elusive manner, so that able observers deny its occurrence. In either case the histological structure of the wall of the gall bladder should be significant, and part of the confusion is due to the fact that this has been a neglected subject. In Koelliker's Gewebelehre, in contrast with almost every other organ, the gall bladder is very inadequately described. Fortunately, Doctor Halpert, with an abundant supply of well-preserved human tissue, plans to remedy this deficiency. Our own observations can therefore be confined to the cat in which, however, the gall bladder is much like that of man.

Three coats are recognized in the wall of the gall bladder—the tunica mucosa, with plicae (consisting of simple columnar epithelium and a vascular tunica propria); the tunica muscularis, and the tunica serosa (B.N.A.). The last of these is thick, and Stöhr, in the 14th edition of his Lehrbuch ('09), subdivides it into a fibrosa, with dense tissue, a loose and sometimes fatty subserosa, and a serosa proper. When contracted, the entire thickness of the gall-bladder wall in the cat as measured in sections (fig. 11) is 2.3 mm. thick, the mucosa (with its tall plicae and thin underlying tunica propria) being 1.5 mm. thick, the muscularis and serosa 0.4 mm. each. If these measurements are compared with those of the strongly contracted intestine of the Guinea-pig, as studied in the Harvard Laboratory by F. P. Johnson,[16] it is seen that the muscle layer is quite thick enough to be an effective agent. In the Guinea-pig's contracted intestine the mucosa forms 75 per cent of the entire wall (as against 67 per cent in the contracted gall bladder of the cat); the muscularis, 18.75 per cent (as against 16.5 per cent in the gall bladder), and the connective tissue 6.25 per cent (as compared with 16.5 per cent in the gall bladder). When the difference in size of the two animals is taken into account, however, these figures show that the muscularis of the intestine is considerably thicker than that of the gall bladder, the difference being far greater than appears, since the fibers in the intestine are so much more closely packed. In both places the circular fibers predominate, although the longitudinal fibers in the gall bladder (apparently more abundant peripherally) do not form a distinct stratum, but occur in bundles interspersed between the circular fibers; and both sorts of bundles are invested by abundant connective tissue.

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[16] F. P. Johnson. The effects of distention of the intestine upon shape of villi and glands. Am. Jour. Anat., vol. 14, no. 2, p. 235, Jan., 1913.

The connective-tissue layer of the gall bladder (serosa) is nearly three times as thick as that of the intestine (chiefly submucosa) and, as stained with resorcin fuchsin, is evidently much richer in elastic tissue; this tissue is surely an important factor in the mechanics of the gall bladder.

When normally distended with bile, the wall of the gall bladder in the cat becomes reduced to at least one-fifteenth of what it was when contracted; and through forced distention, at a pressure of 325 mm. of mercury, even to one-twenty-third, being then 0.1 mm. thick. The intestine, as studied by Johnson, shows no such range of normal change in thickness, nor can it be stretched so far without bursting. In the normally distended gall bladder of the cat, having a wall 0.16 mm. thick, the mucosa measures 0.08 mm., the muscularis and the serosa, 0.04 each. Owing to the presence of elastic tissue between the muscle fibers, the limits of individual fibers are unusually easy to follow, and it seems that in this state the thickness of the muscularis is made up of eight to ten superimposed circular fibers. In the Guinea-pig's intestine, with normal distention, the muscle layer is reduced proportionally somewhat more than in the gall bladder, but in the latter there is a change in thickness from 0.4 mm. to 0.04 mm., showing that there is either great contraction in these muscle fibers or considerable passive stretching (with perhaps a combination of both).

If this musculature expels the bile, it would be expected that such action could readily be obtained by electrical stimulation of the distended bladder, but such is not the case. Doctor Whitaker and I, working together in a series of experiments, found that a strong tetanizing current produced only local and shallow ring contractions on a semicollapsed gall bladder, and had hardly any effect on a distended vesicle; furthermore, continued stimulation never caused a contraction of the gall bladder or expulsion of any bile, although a touch of the electrodes was almost enough to empty the urinary bladder and contract the intestine. The natural supposition, that we are dealing with a specialized form of smooth-muscle contraction which works more slowly or less effectively than that of the intestine, is rendered uncertain by the failure of the wall to press closely on the contained fluid during all phases of collapse, as observed in the living animal when the abdomen is opened. But there is another force at hand, as pointed out by Winkelstein, which is capable of expelling bile from the gall bladder. This is the force of intra-abdominal pressure augmented by respiratory and other movements.[17]

The importance of this external pressure is confirmed by many other authors. Thus Leuret and Lassaigne (from

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[17] Winkelstein, A. (Some observations on the entrance of bile into the duodenum. Surg. Gyn. and Obst., April, 1925, p. 545). If this be the true explanation I would add one other factor. In a fasting animal the ropy bile in the neck of the gall bladder undoubtedly clogs the cystic duct and offers considerable resistance to the outflow of bile. But after meals, and at other times, one may see the yellow hepatic bile invading the cystic duct. It is this observation which has suggested to the writer that it is the backing up of this dilute bile, when the sphincter papillae is intermittently closed, which dilutes the thick fluid in the cystic duct and first makes possible the expulsion of the bladder contents by the available forces, namely, the elasticity of the distended vesicle, intra-abdominal pressure, etc.

Milne Edwards, T. 6) state that they saw bile escape from the outlet of the common bile duct of the horse with every inspiration (through pressure on the liver which lacks a gall bladder and is said to have no effective sphincter papillae). Again, Blondai, working with dogs, saw bile escape from the papilla with vomiting or attempts at emptying the intestines. And in an anesthetized cat, a moment after the liver had been lifted out of the body cavity for inspection, we once had the experience of seeing the dark green bile suddenly shoot down the cystic duct and choledochus into the duodenum, probably as a result of manipulating the liver.

Leaving for a moment discussion of how the bile is emptied by the gall bladder, plenty of evidence remains to prove that it actually does leave the gall bladder via the cystic duct. Often in looking through the transparent walls of the ducts, in freshly opened cats, one may see dark green bile which has extended from the neck of the gall bladder to the duodenum and has even backed up a short distance into the hepatic ducts. Finally, the Meltzer-Lyon test and the injection of peptone (Rost et al.) have proved that samples of bladder bile may be collected from the duodenum following a paralysis or inhibition of the sphincter papillae.

If more evidence be needed that bile flows down the cystic duct, it is only necessary to look at the x-rays presented in plate 1 of this paper. Is it conceivable that in one hour and forty minutes absorption alone could reduce the distended gall bladder of figure 2 to the diminutive shadow shown in figure 3? But granting for the moment that this is possible, why should not a meat diet produce an equal degree of absorption? If one replies that the duodenal sphincter opens less frequently during the digestion of meat than when yolk is fed to an animal, then the larger size of the gall bladder in figure 4, for instance, should be due to the entrance of more yellow bile than occurred in the gall bladder in figure 3. Yet this is not the case, for the bile content of the gall bladder of meat-fed animals (table 2, B) is almost always dark green and inspissated, showing that very little if any new bile enters the gall bladder during the first four hours after a meal of horse meat. Taking all these facts into consideration, which have been derived from observations of living animals, there would seem to be little doubt that in addition to the removal of certain constituents of the bile by absorption the remainder is expelled through the cystic duct following a meal of yolk and cream.

This brings us to a consideration of the mucosa of the gall bladder—the organ of absorption. These experiments have shown that the mucosal pattern undergoes remarkable changes in passing from distention to collapse. But in current descriptions a single pattern is mentioned without reference to the position of the organ in the functional cycle; so that usually it is said to be raised into rugose folds (of greater and lesser magnitude) bounding polygonal spaces which give the interior a honeycomb appearance. This is also the picture one sees upon looking into the distended or semidistended gall bladder of the cat. Apparently, the only change which takes place in the mucosa during fasting is merely the widening or narrowing of the meshes as the pressure fluctuates. To see if these folds could be obliterated, the writer subjected the gall bladder of a living cat to an internal pressure of 325 mm. Hg., far exceeding the secretory pressure of the liver. Yet not even the rugae of lesser magnitude were flattened out (fig. 7). Evidently, the greater abundance of elastic and collagen fibers, as compared with the intestine, prevents the obliteration of the mucosal folds.

But the gall bladder of the cat when completely collapsed presents a very different appearance, the lumen being nearly filled with large fungiform projections (fig. 8) which arise from the intersection of the rugae. For this reason I have called them 'nodal villi.' Some of these are finger-like, most of them globular, and still others compound (see the left-hand side of fig. 8) as if the point of intersection and portions of adjacent rugae had been bodily uplifted. These villi first appear when the gall bladder is about half collapsed. At that time the points where the rugae intersect assume the form of slightly mounded areas. As the gall bladder continues to collapse, these mounds become arched and then protuberant. The arched stage is shown in figure 9, a model of the mucosa of a nearly collapsed vesicle. In this figure the origin of two arched villi from nodal points is easily demonstrable as well as the height to which they rise above the

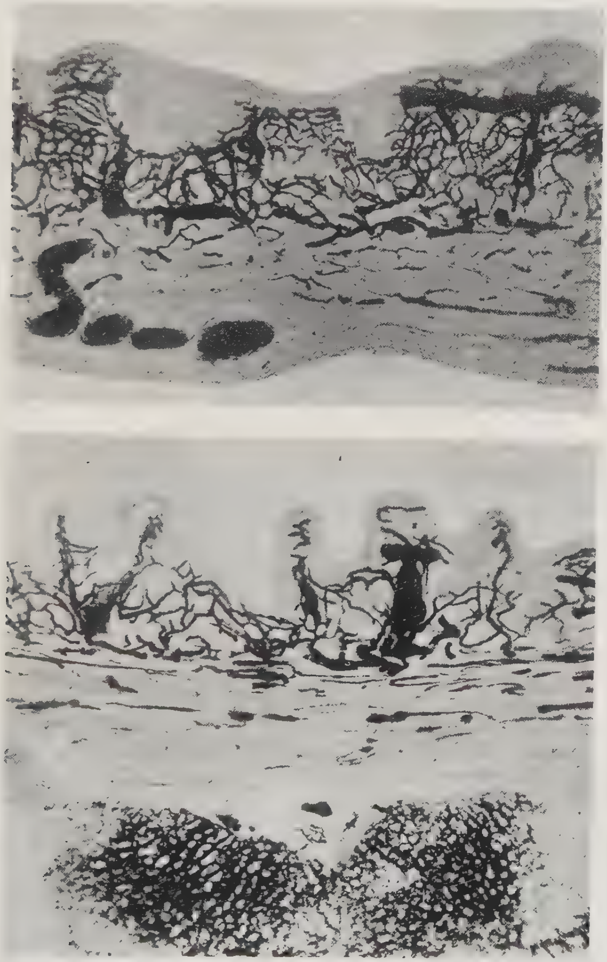

Fig. 1 Longitudinal and transverse sections of the nearly collapsed gall bladder of a cat (no. 2533, table 1) injected with India ink through the cystic artery (cf. fig. 11).

basal pockets. But the most instructive figure is the cross-section of the fully collapsed stage (fig. 11). This is placed beside a photomicrograph of a fully distended bladder (fig. 10), magnified at the same scale, to show the enormous change which accompanies distention. Since the mucosal pattern of a human gall bladder is much like that in the cat when both are partially distended, it is fair to assume that nodal villi will be found in human gall bladders when a fully collapsed stage of that organ has been observed.

This radical change in pattern raises the question as to which condition favors the most rapid absorption—distention or collapse. In the absence of empirical data, one would presume that more vascular surface would be in apposition to the bile when the gall bladder is partially collapsed than when it is distended. Certainly, the capillary network of the rugae would be more extensive in collapse than when the latter were nearly pressed out by distention.[18]

Another issue which should be met is the relative activity of lymphatics and blood capillaries in absorption—as to whether a specificity like that in the intestinal villi is to be expected. Although the literature speaks almost exclusively of lymphatics in dealing with the function of absorption, figure 1 of a partially collapsed gall bladder which has been injected with India ink through the cystic duct reveals a profuse network of blood capillaries which should not be without significance.

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[18] This question was suggested by the peculiar histological appearance of the larger villi (fig. 11). A close examination of the photomicrograph shows that throughout the plicae the tunica propria is of uniform density except at various points along the free surface where the villi form. Here the intercellular spaces are distended with a substance staining like the bile in the adjacent lumen of the gall bladder. This substance accumulates to such an extent in the villi that the overlying epithelium becomes quite thin and the intercellular spaces are enormously distended, suggesting an artifact. Neither the lymphatics nor the blood vessels seem to be involved in this distention. It is not believed, however, that any postmortem change could have taken place, as this specimen was transferred directly from the living animal to Zenker's fluid. Accordingly, it may represent a phase of absorption characteristic of the collapsed gall bladder, requiring special investigation.

CONCLUSION

The reader familiar with anatomical history has doubtless been aggrieved by my failure to cite early authorities, often pertinent and weighty. In part this has been intentional, since the problem of the gall bladder is as old as scientific medicine and has a boundless literature. It is surprising how much of what is now written on the subject has already been said.[19] Galen knew that gall bladders were not of one size, but found them in his dissections of animals ''sometimes very full, sometimes empty and sometimes in an intermediate state'' (De nat. facultat., Lib. III, Cap. V). He argued that their contents entered and left at different times through one and the same passage. If that passage seems to Halpert to offer mechanical obstructions, Glisson has already noted the low position of the fundus; and all unfavorable folds and bends in the passage have been the subject of repeated comment. Haller describes fairly well the muscle coat of the gall bladder and recognizes the indolence of its contraction—''surely less lively than the urinary bladder''; but he is confident that bile is discharged from the gall bladder into the duodenum. In animals of steady habits (the Herbivora, many of which have no gall bladders) he finds that the rigid necessity for more copious bile at the time of eating would not be such as to require a special receptacle, and he brilliantly records ''a suspicion that those animals which never eat fat are not so much in need of acrid bile as those which take fat with their food.'' The concentration of bile in the gall bladder led Diemerbroeck to debate whether there are not 'two biles,' so different are the fresh hepatic product and the contents of the vesicle; and the effect of intra-abdominal pressure on emptying of the gall bladder, as discussed by Winkelstein in 1925, recalls that Diemerbroeck (1672) saw in the pressure of the full stomach the timely agent for evacuating the gall bladder when its contents would be most needed.

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[19] Working in the same laboratory with Prof. F. T. Lewis, who is much interested in the historical aspects of anatomy, I have embodied in the following section certain of his comments.

Finally, the account of the mucosa by Boyd in 1923 (Brit. Journ. Surg.) who found the sight 'a beautiful one,' as if he were ''gazing into the depths of a marine pool at seaweeds and sea anemones,'' has its counterpart in the early description of Haller, who defined the mucosal pattern as 'prettily' reticulate (pulchre reticulata) and the raised interconnections as short, mutually entangled, with pits in between. ''Free villi (floccos) I have seen, but not often.''

With a selection of the deliverances of the early anatomists the conclusions which I have reached are in accord—more so than with some recent opinions. The gall bladder fills with bile which is there concentrated or otherwise modified, but is not altogether absorbed. One hour and three-quarters after the ingestion of a meal of lipoids and fats (egg yolk and cream) it discharges its content through the cystic duct, to aid presumably in the digestion of that food, leaving a collapsed bladder of very different histological character. After approximately five hours, it slowly refills. Direct observation of this process leaves the impression that the gall bladder is a passive agent in this cycle of events—except for the elasticity of its wall—the active factors being the secretory pressure of the liver, the regulatory action of the sphincter papillae, and intra-abdominal pressure. But operative procedures now in progress suggest that the gall bladder is not wholly inactive and that the solution of this problem requires more subtle methods than have hitherto been employed.

PLATES

PLATE 1

EXPLANATION OF FIGURES

Cholecystograms of the human gall bladder

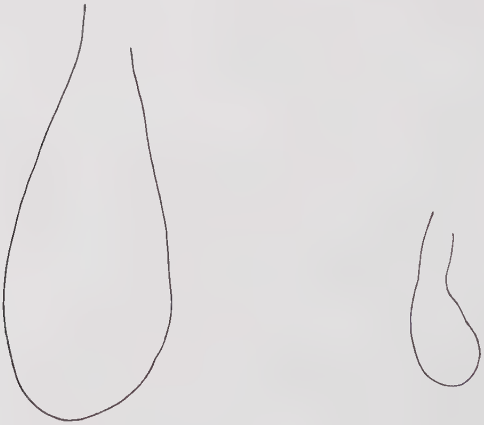
2 A distended human gall bladder, during period of fasting, seventeen and one-half hours after taking twelve pellets of the sodium salt of tetraiodophenolphthalein. Note position of gall bladder between second and fourth transverse processes of lumbar vertebrae.

3 The same gall bladder, one hour and forty minutes after ingestion of a half pint of cream and the yolks of four raw eggs. Note position of gall bladder between second and third transverse processes.

4 The same gall bladder, one hour and forty minutes after eating 9 ounces of rump steak. Note position of gall bladder between first and third processes (cf. fig. 3).

5 The same gall bladder, three hours after the meal of steak. Note position of gall bladder between second and third transverse processes.

6 The same gall bladder five hours after the meal of steak.



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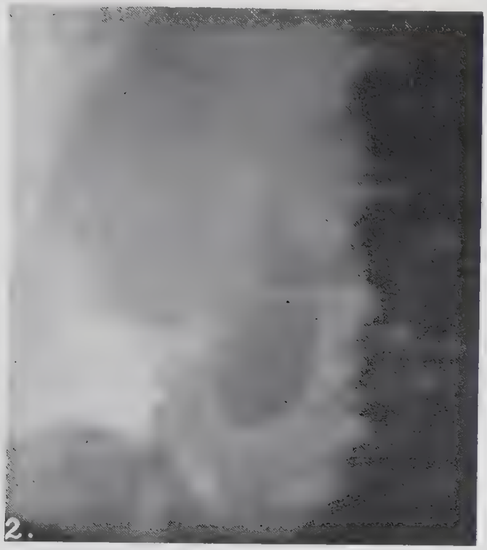
3 a

Pen-and-ink tracing of gall-bladder shadows shown in figures 2 and 3



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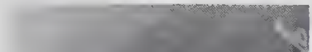

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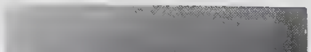

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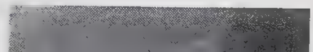


6

PLATE 2

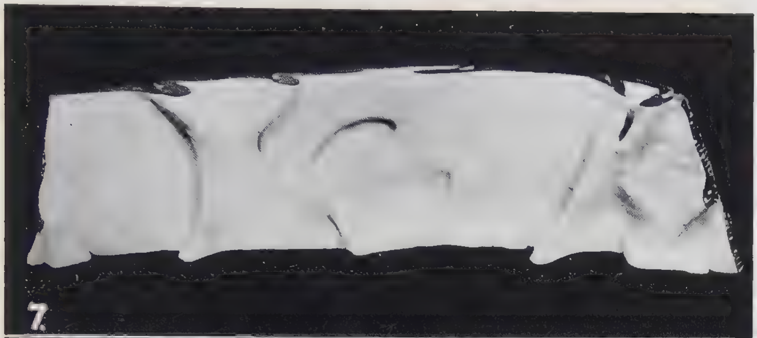
EXPLANATION OF FIGURES

Models and photographs of the gall bladder of the cat

7 A wax model of a portion of the mucosa of a distended gall bladder artificially dilated to a pressure of 325 mm. Hg (no. 2524, × 72). Note that neither the rugae of larger or smaller magnitude are obliterated by this relatively enormous pressure.

8 A photograph of the interior of a gall bladder in the state of maximum collapse, six and one-half hours after a meal of egg yolk and cream (no. 2578, table 1, × 4). Note thick walls and protuberant villi.

9 A wax model of a portion of the mucosa of a gall bladder which was nearly collapsed (no. 2495, table 1, × 112), five and three-quarter hours after a meal of yolk and cream. Note origin of two nodal villi at the areas of intersection of rugae.



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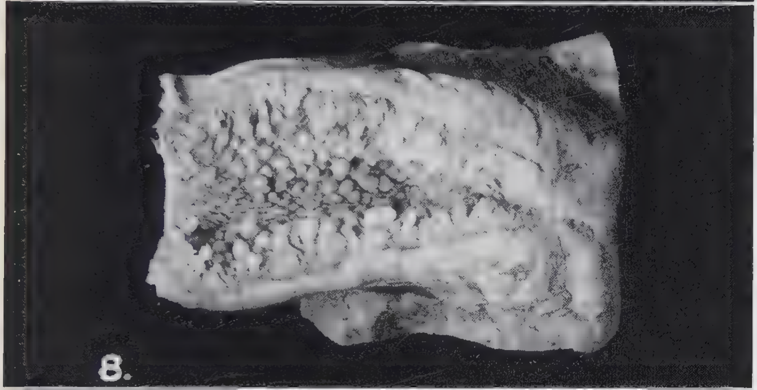

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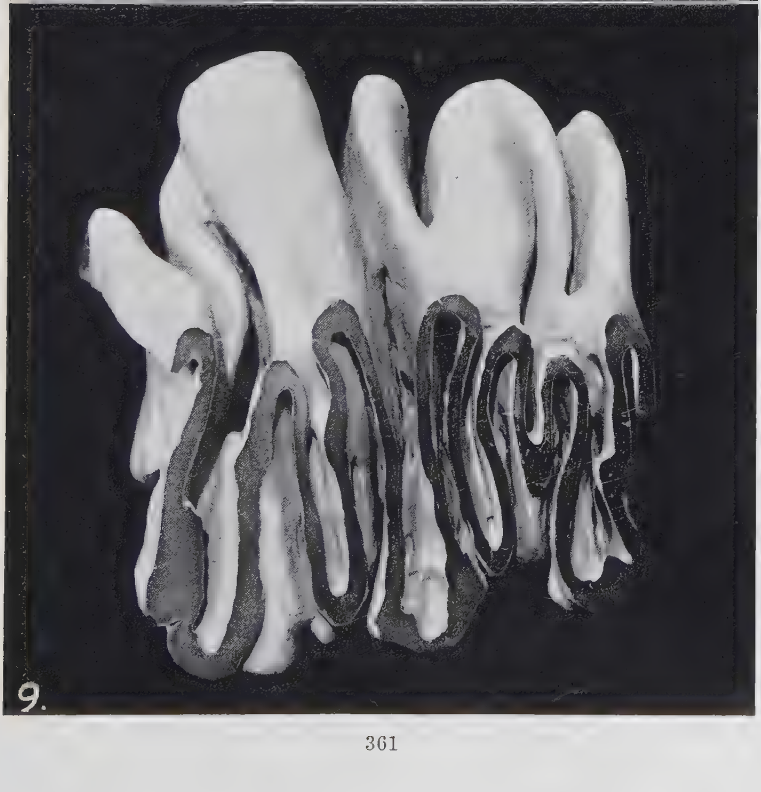

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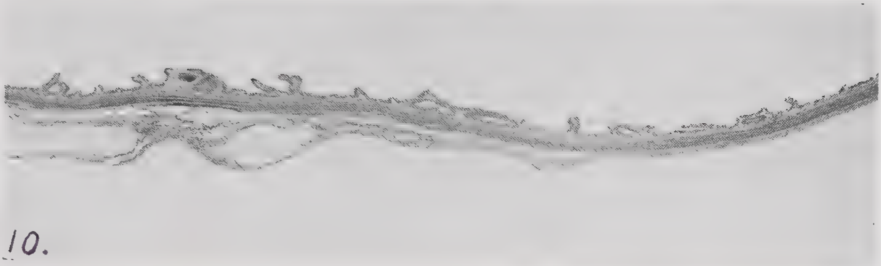
PLATE 3

EXPLANATION OF FIGURES

Photomicrographs of the cat

10 A cross-section of a normally distended gall bladder of a fasting animal (no. 2573, × 29; Mallory's connective-tissue stain, 8 μ). The entire wall, on the right, measures 0.16 mm. in thickness, the mucosa constituting half the thickness (0.08 mm.), the rest being equally divided between the muscularis and the serosa (0.04 mm. each).

11 A cross-section of a fully collapsed gall bladder, six and one-half hours after eating a meal of yolk and cream (no. 2578, × 29; Mallory's connective-tissue stain, 8 μ). In this specimen the entire thickness of one wall measures 2.3 mm., nearly fifteen times that of figure 10, the mucosa constituting 1.5 mm., the other two layers 0.4 mm. each. Comparing figures 10 and 11, it will be seen that in passing from distention to collapse, the muscularis and serosa each increase in thickness ten times, while the mucosa is increasing its thickness nearly nineteen times.



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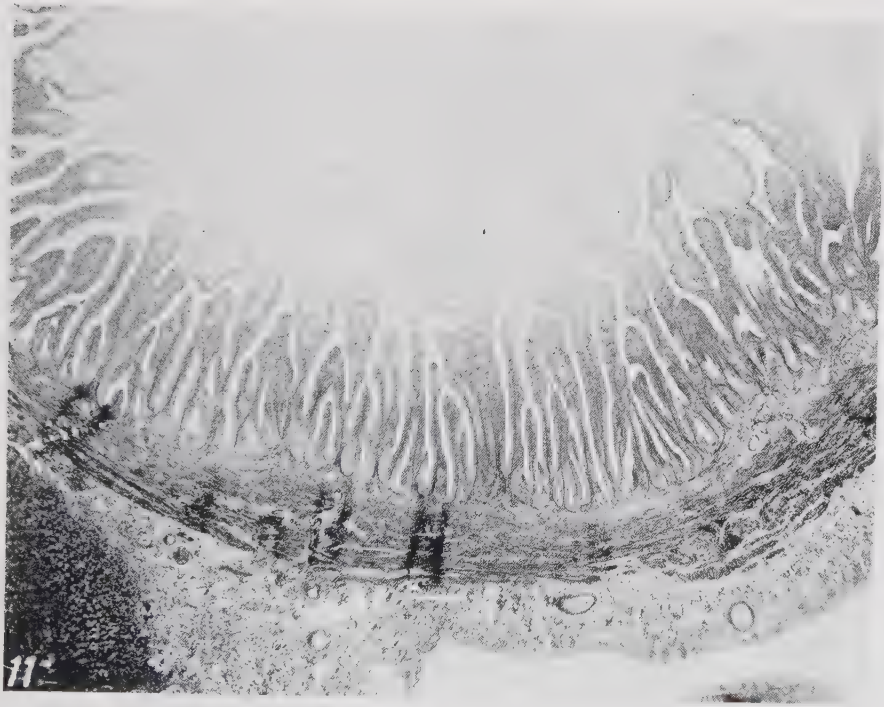

11

ON THE SEXUALITY OF THE RIGHT OVARY OF BIRDS

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ONE PLATE (FIVE FIGURES)

In a well-done experiment Benoit ('23 a, '23 b) was able to show that following the removal of the left ovary of young fowls the new developments which often occur within the atrophied right gonad are of definitely testicular nature. The earlier observations of Goodale ('16), Pezard ('22), and Zawadowsky ('22) on the appearance and characteristics of the right gonad following such unilateral ovariectomy, together with related data from other sources to be mentioned later, were easily capable of resolving this problem in the same sense. It remained for Benoit, however, to observe two additional cases and to demonstrate in both of these the development of true testicular tissue within the unoperated rudimentary right ovary. In clearing up this point Benoit has made an excellent contribution to the large amount of existing data on sex in birds; he has also added an item of importance to the subject of physiological sex reversal and to sex theory.

It unfortunately happens, however, that although he has supplied the plainly necessary description and interpretation of the structure thus produced in the right gonad, Benoit has offered an obviously erroneous 'explanation' of this development of testicular tissue within the rudimentary right ovary. In even the third of his publications ('24) on this subject he sponsors a view which ignores many available facts and which stands in plain contradiction to a number of them. In order that the full value of his main contribution may be utilized, instead of becoming needlessly obscured, it seems that the facts which show the untenability of the explanation offered by him should be pointed out. Also, if possible, a true explanation—at least one that is consonant with other kinds of data on differences in right and left gonads in birds—should be supplied. To do these two things, and to make it clear that the right ovary of birds is really an ovary, is the purpose of the present paper.

Benoit removed the left ovary from two female chicks aged four and twenty-six days. The latter bird was killed when six months old and found to have a testis on the right side and no trace of a gonad on the left side from which an ovary was earlier removed. The bird ovariectomized at four days was killed after five months and the condition of its gonads found to be of similar nature. The modifications of the secondary sexual characters of these two birds are well described in Benoit's first paper ('23 a); some of these secondary characters, together with histological details of the testis structure, are more fully given (with photographs) in the second paper ('23 b). As stated above, a number of earlier observations clearly indicate that a similar change had repeatedly occurred under similar circumstances. Only Benoit, however, unqualifiedly described this development in the right gonad for what it really is and supplied clear and adequate proofs. As to the above facts, no questions need be raised; they are in full accord with several kinds of information already available concerning differences in the right and left gonads of birds. But the explanation which Benoit offers for this development of testis within the rudimentary right gonad must be thoroughly examined.

In addition to the experimental results cited above, Benoit calls attention to the interesting fact that of the twenty-one hermaphrodite birds known to him and previously well described in the literature, eleven had a left ovotestis and no right gonad; ten had an ovary on the left side and a testis on the right side. He also called attention to Firket's ('14) observation that in the embryogenesis of the fowl's right ovary a second proliferation of sex cords does not occur. From these facts Benoit ('24) concludes as follows:

> On arrive ainsi par l'expérimentation et par l'examen des sujets hermaphrodites, à supposer que le glande sexuelle droite de la Poule aurait *la valeur d'une glande testiculaire rudimentaire* (italics are ours). . . . . Ceci nous montre que la glande sexuelle droite rudimentaire de la Poule, qui a été appelée jusqu'à présent "ovaire rudimentaire droit", soit etre considérée comme ayant la valeur d'un testicule rudimentaire, puisqu'elle est formée exclusivement par les éléments de la première poussée sexuelle, et que ces éléments donnent toujours naissance aux cellules de la lignée spermatogénétique, quand ils viennent à se développer.
>
> Ces faits me conduisent donc à considérer que deux germens distincts, mâle et femelle, coexistent dans le même soma, que l'un deux s'est développé et que l'autre est reste potential. Mais il y a lieu de remarquer qu'ils sont aussi rigoureusement fixés l'un que l'autre au point de vue de leur déterminisme cyto-sexuel, et que le germen mâle, bien que réprimé sans doute par la gonade femelle, conserve sa détermination sexuelle rigoureuse, et indépendante des conditions de milieu dans lesquelles il se trouve.

Against this view, that what we know as 'the rudimentary right ovary' is in reality 'a rudimentary testicle,' there is at hand an array of unquestionable facts. It is astonishing that such a view is seriously stated. But since this conception is the central point of Benoit's interpretation, both of his own results and of the peculiar distribution of testicular and ovarian tissue in hermaphrodite birds, it is necessary to set forth these opposed and decisive facts and also to supply additional data. Before proceeding with this task, however, it may be noted that Benoit's idea is essentially a recrudescence of a view stated by Laulanie ('86) nearly forty years ago. This worker thought, as has been already noted by Swift, that the primordial sex gland of the chick was bisexual; that the germinal epithelium produced the female elements, and that the male elements or cells were present in the network of cords which were developed from the stroma in the midst of the gonad. The cortical ovules in the germinal epithelium were female and the medullary ovules were male.

According to this idea, the gonad was neither male, female, nor indifferent, but hermaphroditic. Later on in development one of the sexual elements degenerated and the gland became male or female as the case might be. It could have been thought that this view was merely of historic interest.

First of all, the examination of any considerable number of adult fowls would have made it clear to Benoit that the right ovary of this animal not infrequently forms true oocytes in large numbers—though they usually remain of small size. This fact is conclusive on the main point under consideration. Under undisturbed conditions this formation of oocytes certainly occurs hundreds of times more frequently than testicular tissue appears in this rudimentary gland. Indeed, there is no existing evidence that testicular tissue has ever developed in the right ovary under normal undisturbed conditions. Most of the best-studied cases of hermaphroditism (in which testicular tissue developed on the site of the rudimentary right ovary) give clear evidence of such disturbance; and, surely, the development of testicular tissue following unilateral ovariectomy is associated with a real sexual disturbance—castration generally in vertebrates having particularly notable effects on sexuality. Now the frequent presence in this right gland of eggs in one or another stage of growth at once indicates both the ovarian nature of the gland, and the earlier occurrence within this gland of a 'second proliferation of sex cords.' On his own account Benoit states: "J'ajoute que je n'ai jamais rencontré d'éléments sexuels de femelles dans la glande sexuélle droite"; and he further appears to quote Firket as saying that "La deuxieme poussée ne se produit jamais." This is clearly an error, and one avoidable by the observation of a very moderate number of cases of adult fowls.

Again, if Benoit had not opportunity for personal observations of the sort just mentioned, the literature could have been consulted with clarifying results. His ('68) has described instances of the formation of ova in the right gonad of fowls and pigeons, and referred (p. 16) to still earlier observations of 'Emmert, Wagner and others.' Among others, Gadow ('91) and Chappellier ('13) have since added cases which show that this occurrence is widespread in the various orders of birds. More recent investigators, like the present writer, have doubtless considered the presence of ova in the fowl's rudimentary right ovary—and the variable extent to which the gland is developed—as facts too well known to require special mention. In the case of the pigeon, however, the writer has called attention in several publications (e. g., '17) to the relatively frequent occurrence of developed right ovaries. The degree of this development was there found to be related to other facts—particularly to the initial degree of femininity of the bird—and this supplied a new and special reason for reference to these right ovaries. Also, in connection with a paper dealing chiefly with testis size we supplied data ('18, table 2) concerning the right ovaries of twenty-one doves killed three to seven weeks after hatching. In four of these twenty-one cases the right ovaries were 3 to 11 mm. in length, and unmistakably ovarian in content. Traces of this gonad were present in eight additional cases, and it was doubtless ovarian in all of them, though one does not always obtain conclusive macroscopic evidence of the ovarian nature of the gland in young birds in which it is found in traces only.

Among other birds than fowls, the same conditions—frequent persistence of the gland, the presence of oocytes within it, variable degrees of its development when present—are quite generally found. Data for these birds are not very readily available, however, and at this point we may therefore supply additional quantitative data for various pigeons and for some other wild birds taken in or near the breeding season. In table 1 data are given for only two kinds of adult pigeons (4+ months), and for only the last 200 females of each of these kinds of pigeons killed (at various seasons) by us. In the case of wild birds listed, all of our data obtained between April and October—the period in which a minimum of ovarian tissue on the right side could be best detected—are tabulated.

Different species of birds doubtless vary in the percentage of individuals which thus succeed in the formation of oocytes in the repressed right ovary. Among the individuals of any single species there is also wide variation in the number and extent of the development of these oocytes. The percentage of persistent right ovaries shown by our data for the pigeons and doves probably exceeds the percentage normal to pigeons because of the conditions under which these birds were kept and bred. But in all species of pigeons studied by us, and under any condition of breeding, the right ovary sometimes persists and contains oocytes. In pigeons we have observed numbers of oocytes varying from one to many hundreds, and the size attained has varied from visibility only with a lens to mature liberated ova weighing more than 2 grams.

The tabulated cases show that oocytes, not sperm, are the usual output—when there is an output—of the rudimentary right ovaries of various species of birds. It is already well known that fowls are essentially similar to pigeons in these respects, though the persistence of right ovaries are probably somewhat less frequent in fowls than in pigeons. Oocytes were observed in every one of the cases of right ovary listed by us in table 1. Clearly, 'la deuxieme poussée' and various still later phenomena of ovarian development do occur, and they occur frequently as well. In order to put this development of oocytes in the right ovary more fully on record, the various figures of plate 1 are submitted. The reader is referred to the cases there figured.

It is further notable that any one who has had occasion to observe large numbers of rudimentary right ovaries of birds finds them quite generally, if not quite exclusively, to have the shape and contour of immature ovaries. And the differences of testes and ovaries in these respects in posthatching stages are ordinarily so characteristic as to preclude any possibility of their confusion. It must then be said that in the numerous cases of greater or less persistence of this right gonad it has characteristically the shape, contour, and appearance of an ovary, in addition to its telltale formation of oocytes. We have been able to show conclusively in pigeons that testicular tissue appears in this right gonad only as an extremely rare event (a rarity commensurate with true hermaphroditism) by sectioning those rare cases of right gonads whose appearance suggested the presence or admixture of anything other than ovarian tissue.

The preceding paragraphs show that when the right ovary of birds does not degenerate completely—but persists in one or another of all imaginable degrees of development, except for size—it normally and usually (rare exceptions) presents itself as unmistakably ovarian; either a second, or still later, proliferation of sex cords has thus occurred in these cases in which the development of this gonad was less repressed, or—a most improbable thing—these oocytes were formed from the first proliferation. In either case Benoit's description and conception is in error. In the very restricted number of cases in which testicular tissue has been found and described in the right gonad, probably all were associated with unusual procedures or conditions—ovariectomy; or, both ovary and testis in the left gonad; or, antecedent disease (known or unknown), starvation, etc. It is evident, therefore, that the real problem is to account for the rare and in-

TABLE 1

On the frequency of 'persistent right ovaries' in females of various species of birds

KIND OF BIRD	ADULT OR JUVENILE	NUMBER OF FEMALES EXAMINED	NUMBER OF RIGHT OVARIES FOUND
Common pigeon	Adult	200	19
Ring dove	Adult	200	47
English sparrow (Passer domesticus)	Adult	22	1
	Juvenile	22	6
Molothrus ater	Adult	12	1
Galeoscoptes carolinensis	Adult	27	1
Turdus mustelinus	Adult	13	1
Robin (Merula migratoria)	Adult	21	1
	Juvenile	7	3

duced appearance of testicular tissue in a place where ovarian tissue began its development and where it is expected in case any tissue develops, but where it is known to be always more or less repressed.

So far as the ovaries of the two sides of the bird's body are concerned, the first evident fact is that the left develops freely and the right always exhibits one or another degree of restriction of its development. Probably a complete solution of the cause or basis of this restriction is not now at hand. But is it a matter of indifference to this question that the testes of the right and left sides of the body usually or often show a directly opposite arrangement of free and of restricted growth? In this Journal the present writer has previously published ('16, '18) two groups of data which show that in various species of pigeons the right testis is usually larger than the left; that disease and hybridity both lessen the excess of larger right testes; that the left (persistent) ovary of young squabs is more elongate than the right and thus in form more resembles the left testis than the right testis; and that the chronic diseases commonest in pigeons produce a more profound reduction of size in testis than in ovary. In a later paper (Riddle, '24 b) a long series of known differences in gonad development—of both testes and ovaries—on the two sides of the bird's body have been discussed and additional data given. The reader is referred to the papers cited for an adequate consideration of these matters; but the present discussion must utilize the several facts established in those earlier studies.

Two facts of present interest on the relative size of the right and left testes in pigeons are to be found in the data of table 2. The right testis of the only pure species listed (Streptopelia risoria) is larger than the left in twelve each of thirteen measurements made on healthy adult males. When this same ringdove species is crossed with other species, genera, and families, this great inequality in the number of larger right gonads in the resulting hybrids is progressively reduced—from 9.5 : 1 in closest or nearest related crosses, to 0.8 : 1 in family crosses. It has earlier been shown that the sex ratio obtained from these crosses likewise progressively changes from a nearly 1 : 1 ratio in the pure species (and the closest crosses), to males only in the family hybrids.

The two points shown by the data just mentioned are: First, that the excess number of larger right testes in birds of pure species is no accident—the right side of the body of the male is the side on which testis growth is the more favored. Second, the shifting of the degree of this favorableness runs parallel with the degree to which sex itself—the sex ratio—is shifted or modified. That a similar shift of the sex ratio occurs in crosses of other kinds of birds than pigeons has been shown by the several investigators cited in an earlier study (Riddle, '17). The small amount of data available on testis size in other birds than pigeons would indicate that larger right testes is not the rule among all species of birds; but effects of season, confinement, disease, and hybridity will all have to be eliminated from these meager data before we shall know the extent and value of these apparently exceptional cases (Riddle, '24 b). Further data on various wild species are being collected by the writer.

TABLE 2

Size relationship of right and left testes as affected by hybridity (healthy adults)

KIND OF BIRD	RATIO L — : L +	WEIGHT RELATIONS AND NUMBER IN EACH CLASS					
		Number	Weights (mgm.)		Number	Weights (mgm.)	
			Right	Left		Right	Left
St. risoria	12.0 : 1	36	624	494	3	410	425
Streptopelia hybrids	9.5 : 1	133	571	466	14	465	519
All specific hybrids	6.9 : 1	90	584	492	14	600	654
Complex hybrids	3.4 : 1	131	555	436	39	400	698
Generic hybrids	3.1 : 1	58	476	394	19	412	496
Subfamily hybrids	2.0 : 1	2	392	297	1	253	268
Family hybrids	0.8 : 1	11	475	415	14	390	616

The tabulated data and the facts already mentioned make it quite clear, however, that the matter of repressed ovarian growth on the right side of the bird cannot be adequately considered apart from the large body of data which shows that testicular growth is relatively repressed on the left side. With both of these aspects of the subject in mind, we may now turn to a statement made (p. 87) in our first paper dealing with this subject ('16).

> Firket ('14) and Swift ('15) have shown that in the chick embryo there were more primordial germ cells in the left gonad, and that this (left gonad) is there also distinctly larger than the right. Allen ('07) found that the sex cells were unequally distributed to the two gonads of the turtle, the left receiving most. In this form only 24-70 per cent of the sex cells ever enter the gonads. . . . . The meaning of this pronounced inequality in the distribution of the primordial germ cells which is plainly associated with a larger left embryonic gonad . . . . is by no means clear. But, whatever this may mean, it is probably a situation of importance to the theory of sex.

This discovery of a large excess of primordial germ cells in the left gonad has been duly considered by us in three later studies ('18, '24, '25) concerned with one or another aspect of the differential development of the gonads on the two sides of the bird's body. Any and all discussions of this point must take into account the series of facts made available in the several publications of Firket and Swift. Both Firket ('14, '20) and Swift ('14, '15) made the following series of very significant observations on the 'primordial germ cells' of the chick: These cells arise in the extra-embryonic area anterior to the head fold; they early enter the embryo by way of the vascular system; they are thus early distributed to practically all parts of the embryo; they later proceed—again within the blood vessels and perhaps also by amoeboid movement—to the region of the two genital ridges; and *two to five times as many of these cells find lodgment in the left genital ridge as in the right one.* This last-named point has been recently examined and confirmed by Defretin ('24).

From these studies it is clear that primordial sex cells of two distinct cytosexual constitutions in the right and left germ glands of the same bird is particularly unthinkable in the case of the bird, for in this animal (chick) these cells reach the embryo by way of the circulation, and they *circulate* within the embryo prior to their lodgment in the right and left anlagen of the gonads. These conditions therefore afford unusual assurance that normally the cytosexual constitution of the two gonads of one and the same bird are qualitatively alike. Even a qualitatively unlike division in the formation of the first two blastomeres would here fail to provide the two sides of the body with primordial sex cells of different constitution. Such two blastomeres, however, might or might not provide the peritoneal cells (germinal epithelium) of the two gonad areas with an unlike constitution, and, according to Swift ('16), it is not entirely certain that oocytes and spermatocytes both arise from primordial cells, and not from peritoneal cells. In any case, the peritoneal cells of the two sides and the primordial cells of the two sides of any one bird must be regarded as normally possessing identical chromosomal equipment.

The above considerations indicate that the last paragraph of our quotation from Benoit is a statement wholly apart from the known facts and conditions. The known facts contradict the assumption that cells of a male cytosexual constitution are laid down in the right gonad of the female, and that these rigorously conserve this constitution independently of their surrounding conditions and against the repressing influence of the left ovary. Indeed, these facts all point to 'changed conditions' as the precise means of arriving at a male type of gonad from a cytosexual constitution which normally results in a female type of gland. And, as already noted, probably no single case of the development of testicular tissue within the rudimentary right ovary of an undisturbed and sexually normal bird has yet been observed.

On the other hand, we do know that some sort of very early difference existing at the site of the right and left genital ridges does effect a numerically unequal distribution of these primordial cells. Firket and Swift both think that chemotaxis is probably involved; the former investigator also figured and described ('20) the anatomic relations of the two genital ridges at this period. Whatever the nature of this initial difference may be, still another or further difference probably results from the unequal reception and content of primordial sex cells. Doubtless, it is these initial inequalities which explain the fact that even the right testis of the male remains smaller than the left during the early embryonic stages of the chick, as was found by Swift ('16). The larger ultimate size of the right testis is thus attained despite a situation which has the appearance of an initial handicap. We have elsewhere (Riddle, '25) pointed out that this unequal distribution of cells also provides an intelligible basis for the observed fact that the right gonad is more often completely absent (in males, and in female embryos) than is the left gonad. If one or any minimum number of these primordial sex cells is essential to gonad development, this minimum number—on the basis of chance—will be lacking more often on the right than on the left side of the bird's body. In any case, these primitive quantitative inequalities of the two gonads are the facts upon which must rest considerations of gonad inequality at any later stage.

The relation of cases of avian hermaphrodites to the present subject may next be considered. The preceding discussion has shown that on the right side of the body of the normal bird testicular development is favored and ovarian development repressed; on the left side testicular development is relatively somewhat repressed in postembryonic stages and ovarian development is favored at all life stages. Let us now observe that the cases of testicular development following ovariectomy and all of the sixteen cases of hermaphrodites earlier cited by us ('24; ten of these first cited by Benoit) which had gonadal tissue on both right and left sides are in every instance examples of this same rule—favored development of testicle (whether in male or female) on the right side.

But very new light has recently been thrown upon these cases of hermaphrodite birds. The data of Crew ('23), Fell ('23), and Riddle ('24 a) leave little room for doubt that the cases of hermaphrodite birds earlier described, excepting possibly those also 'bipartite' in plumage, are really cases of incomplete sex-reversal—cases in which the character and product of the sexual glands are changed under functional stress and without change in their cytologic constitution. The last-named author, in particular, has directed special attention (pp. 178, 179) to the fact that the cases of complete sex reversal observed by Crew and by Riddle now make it entirely probable that the so-called hermaphrodites were in fact stages in sex reversal. There is now no other way of regarding these hermaphrodite birds, without at the same time disregarding the most significant facts learned from the best-observed cases. Benoit is right in considering that the facts obtained from unilateral ovariectomy and from avian hermaphrodites should be treated together. He failed to recognize that both of these facts must also be considered in relation to the several additional facts which have been brought together in the present discussion.

One can further agree with Benoit that the rather usual failure of a second proliferation of sex cords in the right ovary is a matter worthy of note. But how may one think of a second proliferation of sex cords as proceeding from, or giving rise to, cells of different cytological constitution than those of the first proliferation? Such a conception is based on no known fact. Moreover, we have shown above that such second proliferations (at least the formation of ova) do frequently occur and under circumstances which must be considered essentially normal. Neither such frequency nor such normality can be declared for any one of the extremely rare cases of the origin of testicular tissue in the rudimentary right ovary.

The more usual rule of failure of a second proliferation of sex cords seems, however, to have a far simpler significance than that ascribed to it by Benoit. It is doubtless to be regarded as merely one of many evidences of an early check, halt, and degeneration within the right ovary. More commonly, without doubt, a complete halt comes in the fowl and in some other birds only after the first and before the second proliferation; but, as we have seen above, it frequently passes through both, and with diminishing frequency it attains to progressively later stages of ovarian development. Swift ('16) has shown that the sex of the chick embryo (six and one-half days) can be identified immediately after the formation of the first proliferation of sex cords; it is thus seen that when cytosexual constitution is really involved, this first proliferation leaves male and female gonads in an already dissimilar and characteristic state.

One further and obvious point concerning the more ready development of testicular tissue in the right than in the left ovary remains to be mentioned. In all cases the differentiation of ovarian tissue has proceeded less far in the right ovary, and this restriction upon growth and differentiation begins quite early—during the embryonic history. This tissue, less differentiated than that of the unrestricted left ovary, should therefore be more capable of completing its differentiation in another direction—as testicular development—than is the more highly differentiated tissue of the left ovary. This is merely an acknowledgment of the general principle or 'law of genetic restriction' which is applicable in embryogenesis generally. This fact and other facts already reviewed should assist in a comprehension of, though they do not fully clarify, the circumstance observed in many divergent aspects of sex research in birds, namely, that of the two points at which the germ glands develop in the bird's body the growth of testis is more favored, and the growth of ovary more repressed, on the right side.

It seems that even Benoit has given so limited an examination to the facts of physiological sex theory developed during the last fourteen years that he still feels obliged to try to fit his own observations—which are made upon a much modified sex situation—to the facts of chromosomal influence on sex under undisturbed conditions. He is thus led to views and assumptions which can have no foundation in fact, which leave out of account many available facts concerning the difference in the right and left gonads in birds, and which fail to realize that complete sex reversals have already been effected at both earlier and later stages in the life-cycle of birds.

From the foregoing examination of Benoit's results in relation to the facts necessary to their interpretation, it becomes evident that the development of testicular tissue in the rudimentary right ovary of birds, following removal of the left ovary in the young bird, is a contribution to two aspects of our knowledge of sex in birds. First, his results reveal an additional kind of evidence that the two sides of the bird's body are not equally favorable for the growth of testis and ovary. Second, his results provide cases of sex reversal induced by treatments applied during postembryonic but growing stages of the life-cycle in the bird.

All earlier successes in sex reversal in birds would of course render it probable that sex reversal at this growing stage is possible under proper and adequate procedures; but hitherto such reversals in birds had been limited to still earlier stages (Whitman, Riddle) and to those occurring still later in adult life (Crew, Riddle). The means by which the earlier cases of sex reversal were effected were of course various, though the present writer has been able to supply evidence that all of the means hitherto employed really produce a common effect—a change in the metabolic rate. Does the newly observed method of affecting sex reversal act in the same way? This question cannot now be definitely answered. If it should later be shown that the presence of the left ovary in the young developing female is associated with a lower metabolism than that which becomes established in the unilaterally ovariectomized female, this question would be affirmatively answered. Also, if it merely be definitely proved—and there is already some evidence—that the effects en masse of ovary and testis within the developing body of the bird are antagonistic to each other (even though neither should be found to affect the metabolic rate), the problem may be resolved on a basis not opposed to this view; for, because of some reason now unknown—though apparently related to very localized place or position—testicular development is more favored on the right side of the bird's body than it is on the left.

SUMMARY

Earlier data and additional data supplied here show conclusively that the right ovary of birds frequently persists in fowls, pigeons, and many or most other birds, and that these persistent glands are normally ovarian in character.

The development of testicular tissue within the rudimentary right ovary of female chicks unilaterally ovariectomized when young was adequately demonstrated by Benoit; but this result supplies no evidence whatever that this gland is composed of cells with the cytosexual constitution of the male. The cases of hermaphrodite birds cited by Benoit also supply no evidence of any such chromosomal difference. This author's interpretation of the rudimentary right ovary of birds as a rudimentary testicle is an obvious error.

The cases of testicular development within the right ovary following the removal of the left ovary, as observed by Benoit and by his predecessors who failed adequately to evaluate their results, are cases in which sex reversal has been enforced upon the growing bird. Probably this sex reversal is here assisted by the circumstance that testicular tissue develops favorably, and ovarian tissue unfavorably, on the right side of the bird's body.

The wrongly interpreted experiments of Benoit contribute to physiological sex theory at two points. First, by providing an additional kind of evidence that the two sides of the bird's body are not equally favorable for the growth of testis and ovary. Second, by adequately demonstrating sex reversibility in the bird by a new means applied at a hitherto untested stage in the life-cycle, namely, during growth in postem-

bryonic periods. In earlier work these reversals were limited to means applied at still earlier stages (Whitman, Riddle) or during the later adult life (Crew, Riddle) of the bird.

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PLATE 1

EXPLANATION OF FIGURES

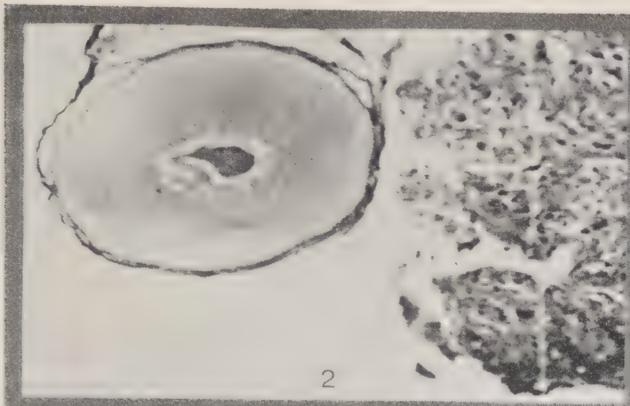
1 Section through entire right ovary of a ringdove (♀108) dead of tuberculosis at 14.8 months. The left ovary weighed 0.155 gram; the right, 0.010 gram (8.8 × 2.2 mm.). This female hatched from the fortieth egg (laid 8/14/14) produced during the season by a Steptopelia risoria female. No. 108 laid twelve eggs, the first at the age of 6.9 months. Numerous oocytes throughout the ovary. (Microphotograph, very low power.)

2 Section of right ovary of a ringdove hybrid, dead of tuberculosis at 11.8 months. Laid first and only egg at 8.5 months. The right gonad was one-fifth the size of the left (neither normal). The single oocyte shown has a diameter of about 1 mm. About ten additional, though much smaller, oocytes (all less than one-sixth diameter of the one shown) discoverable in sections of this ovary. (Microphotograph, very low power.)

3 Section of right ovary of a ringdove hybrid, killed apparently healthy at 14.7 months. This bird produced only two (single clutch) eggs—the first at age of 7.3 months. No eggs produced during last 188 days of life. The left ovary weighed 0.0959 gram; right very small, but with numerous oocytes minute but visible to eye. (Microphotograph, low power.)

4 Section of right ovary of ringdove (Streptopelia risoria) killed, healthy, at 10.4 months. Had laid four eggs, the first at 9 months. The left ovary weighed 0.490 gram; the right, a trace only. The minute oocytes of this right ovary were visible with a lens only and would escape observation by any other than a practiced observer aided by a lens. (Microphotograph, low power.)

5 Section of the right ovary of a virgin generic hybrid (risoria-alba-douraca-orientalis) killed at 6.6 months. Bird thought healthy, but liver and spleen found enlarged. The left ovary weighed 0.126 gram (diameter largest oocyte, 2.4 mm.); right, a trace only with three minute oocytes visible with lens only. Only four oocytes discoverable in sections. This right ovary would have escaped any except practiced and lens-aided observation. The Wolffian and other tubules in this and preceding figures afford further comparisons on size of oocytes. (Microphotograph, low power.)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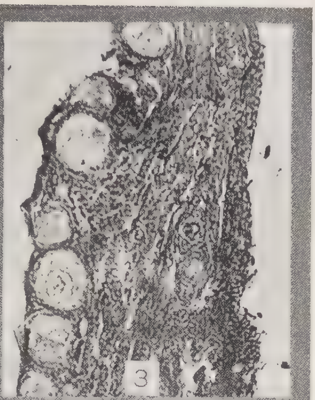
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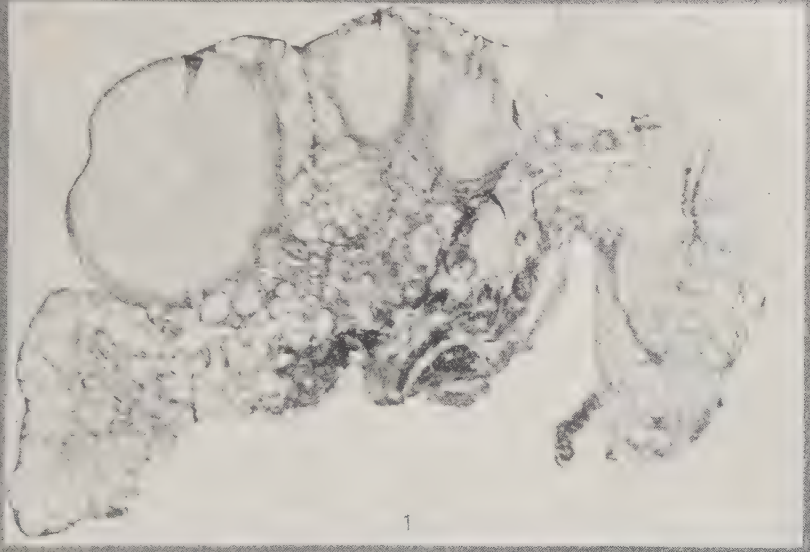
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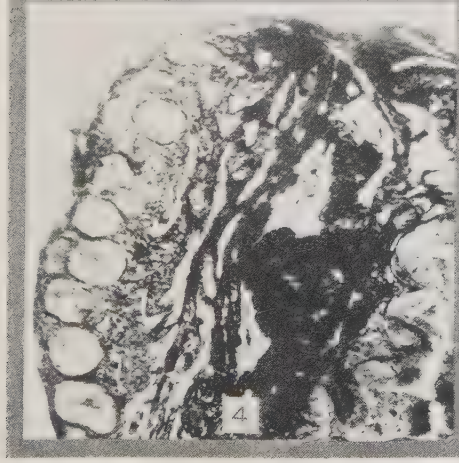
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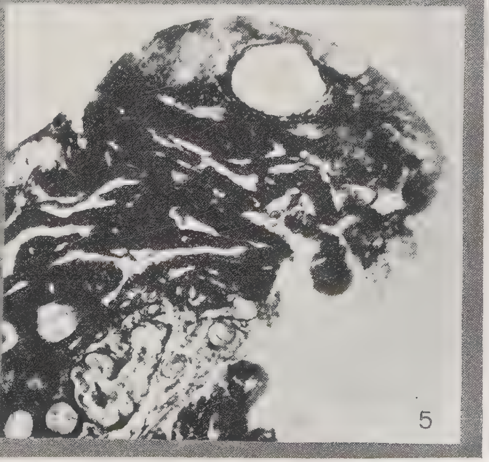
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DEVELOPMENT OF THE EGG OF GALLUS DOMESTICUS IN VITRO

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ONE PLATE (NINE FIGURES)

INTRODUCTION

It is a curious fact that only a few investigators have studied the development of the bird's egg in vitro. Of these the first to be mentioned is Preyer ('85), who demonstrated, in his well-known work on the physiology of the embryo, the possibility of studying the development of the chick outside its natural environment. Preyer removed the fertilized egg from the shell and placed it in a little glass dish which had already been treated with thymol to prevent infection of the white of the egg. Development could then be followed till the end of the second day. Béguelin (1757), whose work preceded that of Preyer's by a quarter of a century, studied the living germinal disc, at intervals, through a hole in the shell at the thick end of the egg. When the embryo was not under observation, the opening was covered with a piece of shell from another egg. Obviously, in this experiment of Béguelin, there is no mention of a development in vitro, and under such conditions his observations could not be very exact. Among the investigators who followed Preyer were Féré ('00), Loisel ('00), and Paton ('11). These authors adopted the method of placing the contents of the egg shell in glass dishes in an incubator and were able to follow developmental processes to a limited degree. In 1912 McWhorter and Whipple ('12) removed the blastoderm of the chick and studied it in vitro, but did not attempt to transplant the whole egg.

The principal difficulties which must be overcome in such experiments are infection of the white of the egg and drying up of the egg, it being the tendency of the yolk to rise above the surface of the liquid in which it is enveloped. The latter can be prevented by adding to the white of the egg that of another egg or a physiological salt solution. In repeating the experiments of the above-mentioned investigators, we modified them in order to get a method by which the development of the embryo could be followed for a long period without interruption. For no matter how excellent the technique developed by Gerlach ('84) may be, by which one can study the embryo through a window in the egg shell, there are certain objections to it. In the first place, one can only study the embryo for a short period at a time, as otherwise there is a danger of suffocation, since no air can penetrate to the vessels of the vascular area through the window. That this method, in the hands of a good experimentist, can give excellent results is apparent from the experiments of Gräper ('11), and in certain cases one cannot say that the method of Gerlach should not b eemployed. But it is never possible in using his method to leave the embryo in perfect rest during the experiment. To insure this, we have taken up the study of the development of the bird's egg in vitro, a short summary of which is given below.

MATERIAL AND TECHNIQUE

For the experiments fresh fertilized eggs of Gallus domesticus are used. Before removing the shell it is necessary to sterilize it thoroughly. This can best be done by following Gerlach's method of placing the egg in a solution of 3 per cent carbolic acid. Experience has taught us that a submergence in this solution for two and a half minutes is sufficient. To avoid contamination in handling, the drying of the egg shell can best be done with the aid of a warm air current (Föhn apparatus). The egg should then be held between the thumb and the index-finger of the left hand so that the equatorial zone of the egg may not be touched. It is even better to place the egg on three upright glass rods, so that it is then supported in three places. Within a few minutes the surface of the egg is dried by the air current. In some cases a little drop of fluid hangs stubbornly on the under surface of the egg. This can be displaced by a small piece of cotton wool, kept in a solution of phenol, which has been cut out beforehand. After the surface of the egg has been entirely dried, the egg should be cut open with a pair of small curved scissors, the initial opening being made with a needle. The cut must be in the equatorial zone. On the top side of the egg a small piece of the shell is left intact. In cutting the egg open it is best to hold it in the left hand following the directions given above. Care should be taken not to rotate the egg during these manipulations, in order to keep the germinal disc on the upper side of the yolk. After the cut has been made, the two parts of the shell are withdrawn in opposite directions, thereby permitting the contents of the egg shell to fall into a sterile glass dish. Half-globe dishes with an internal diameter of about 8 cm. were used in the experiments. The quantity of the albumen of a single egg is not sufficient to cover the yolk. It is therefore necessary to add the albumen of one or more eggs or to use a physiological salt solution. Whenever a part of the yolk is uncovered, and it is of course always that part which bears the germinal disc, a further development is impossible. The addition of the albumen of another egg does not hinder the development of the embryo.

Since, at certain times during development, the specific gravity of the yolk mass is lighter than that of the albumen, it is impossible to prevent the yolk from floating by adding more albumen, and therefore a liquid of a lighter specific gravity must be used. For this purpose a salt solution of the following formula was used: 1000 cc. water, 3 grams of potassium chloride, 3.59 grams of sodium chloride, 0.31 gram of calcium chloride. The added salt solution covers the yolk and the albumen with a thin perfectly transparent layer. As the egg is practically covered only by the salt solution, it is possible to see the finest details in development. In order to prevent too great an evaporation of the fluid and an infection of the culture medium it is necessary to cover the dish with a glass plate. But when the covered dish is placed in the incubator, large fluid drops collect within a short time on the under side of the plate, making continued observation impossible. In order to avoid this difficulty, an incubator was chosen which opened from above. In the lid of the incubator a circular opening was then cut, of such a size as to permit the dish to be lowered through the opening and yet be supported by the edge of this opening. The glass plate which covered the dish, was then wired with a resistance coil and connected with an electric current. By keeping the temperature of the glass at a slightly higher temperature than that of the contents of the dish, the dimness of the glass caused by evaporation was overcome. The wires were so attached as not to interfere with the exact observation of the development of the egg. While the photographs were being made, the heating wires were put aside and afterward replaced on the lid.

In taking photographs allowance had to be made for the upward movement of the egg following progressive changes in the specific gravity of the yolk. This was accomplished by attaching the camera to the tube of a microscope and varying its position above the egg by means of the adjustment screws of the microscope. By this means it was possible to study and photograph the egg without moving or jarring the preparation. This is of special importance in solving the problems which are met with in studying the development of the egg in vitro.

RESULTS

The most important change in the egg to be seen in the first twenty-four hours of life is a definite increase in size of the germinal disc, which gradually continues for the first few days, so that at last the blastoderm covers a great part of the surface of the egg. Figure 1 shows an egg which has been developing for twenty-two hours and ten minutes in vitro. Figure 2 represents the same preparation twenty-six hours later. In the first photograph the attention is specially drawn to the strongly developed germinal disc. Further, both chalazae can be clearly seen and also the albuminous zone which is around the egg. Surrounding this is the salt solution which is perfectly transparent. Besides the great transformation of the germinal disc, attention is specially drawn to an increase in size of the egg which can be particularly remarked by a visible tension of the yolk membrane. While this shows a number of small wrinkles in the newly opened egg, these disappear after several hours. One has the impression that the yolk membrane is greatly stretched. This is particularly well shown by a number of small folds radiating from the point of attachment of both chalazae over a small portion of the surface of the yolk membrane. It was not possible for us to determine precisely the exact increase in volume of the egg. It was too slight to be made visible on the photograph.

Figure 2 shows the special case of a blastoderm growing out in ring form without the development of an embryo. Such deviation from the normal development can also be found in other circumstances and has already been seen by others. The ring-form germinal discs are formed, according to Schimkewitsch, by an unequal growth of the central and peripheral parts of the blastoderm while the anlage of the embryo is degenerating. From the researches of Schimkewitsch ('02) it is clear that the germinal disc is much more resistant to harmful influences than the embryo itself. This is also true of the development in vitro. One can repeatedly see the development of a large germinal disc, while the embryo is hardly being developed at all. It is very probable that the development of the embryo was disturbed by elevation and drying of that part of the surface of the egg which contained the embryo. It was not possible for us always to avoid this.

Figure 2 also shows another remarkable regular feature of these preparations, namely, a sort of cleavage of the yolk mass. The rents in the preparation show a radiating course. These rents are undoubtedly formed by the regular increase in volume of the egg by the absorption of liquid from the surrounding media. The absorption probably takes place over the whole surface of the egg. It follows, therefore, that the yolk mass cannot fill the whole place within the yolk membrane, so that a sinking of the heavier yolk elements takes place. Looking at the egg from the side, one can see clearly a separation of the contents into two layers. Underneath, the yellow, non-transparent yolk mass and above, a broad layer of an opalescent liquid. The constitution of the latter is being studied further. The rents in the yolk mass appear for the first time after absorption of liquid from the surrounding fluid. It is the first sign of the sinking of the yolk substance.

That the development of an embryo in vitro is possible can be seen from figures 3 and 4, which are taken from the same preparation. These were made before we made use of the photographic apparatus described above. That is the reason why both representations of the egg are not in the same position. In figure 3 the large embryo is clearly visible. One sees both cerebral vesicles and the extremities. This embryo, photographed at the end of the fifth day, showed typical spontaneous movements, especially a stretching of the body from time to time.

Very remarkable was the fact that great changes in temperature in the surroundings were not harmful. Thus, some hours before the photograph was taken, some liquid of a temperature of 18.3°C. was added to the preparation which had a temperature of 38°C. in order to prevent the drying up of the egg, a part of which was lying above the surface of the liquid. As a matter of course, the temperature of the perparation became much lower, with the result that the frequency of the heart beat was very slight. It sometimes seemed that the heart was not beating any more. After about half an hour it had recovered its normal movements and beat as frequently as before.

In examining figure 4 attention should be drawn in particular to the peculiar shape of the yolk, which is constricted into two halves, the embryo lying on the smaller portion. The greater part of the vascular area is also lying here. This can be more clearly seen in figure 3, where the smaller lobe is subdivided into three sacculations, the embryo lying on the boundary between these and the rest of the egg. The cause of these sacculations is the great increase in tension of the yolk membrane accompanying the increase in size of the egg. At a certain moment the tension becomes so great that the yolk membrane cannot resist it any longer and ruptures. Probably in our case several ruptures were formed, thus creating the complex hernia shown in figure 3. By the further increase in volume of the egg the remaining pieces of the yolk membrane which separated the hernias from each other would be ruptured, thus giving rise to the large hernia in figure 4. This rupture of the yolk membrane was a regular occurrence in our experiments, and suggests that the formation of a hernia has a direct connection with the osmotic pressure of the culture medium. This will be investigated further.

The photographs in figures 5, 6, and 7 are all taken from the same preparation and show very clearly the formation of a hernia. This process has special significance, as it exercises a harmful influence on the development of the embryo, as does the great increase in volume which the egg undergoes within a short time after the first appearance of the hernia. Figure 5 shows the preparation after ninety-two hours' life in vitro; figure 6, twenty-nine hours later; figure 7, still thirteen hours later. One can clearly see the enormous increase in volume of the egg.

One of the most remarkable appearances that can be seen in the early formation of a hernia is the abnormal development of the vascular area. The blood islands, as it were, are pulled away from each other. No typical vessels are formed. The embryo, which is clearly visible on the photographs 6 and 7, has developed in quite an abnormal way.

In order to be certain that the development of a hernia is the direct result of a rupture in the yolk membrane, we have made experiments with eggs that had developed for some days under perfect normal circumstances in an incubator. By careful opening of the egg shell after this had been bored on the wide end by a needle, it could be clearly seen that the formation of a hernia had also taken place here (fig. 8). The embryo with the whole vascular area lies here on the hernia. One sees clearly the curled edge of the ruptured yolk membrane. The following figure (9) shows another egg which exhibited no sign of hernia when the shell was opened. But upon pricking it with a fine needle it was possible to produce a rent in the yolk membrane. Within a few minutes a large hernia was formed similar to that in figure 6. The position of the vascular area in both cases is the same. It is not possible to explain why the hernia is formed much faster in the latter experiment than during the development of the egg in vitro. Probably, in the latter case, the rent is not formed so quickly.

SUMMARY

According to the technique described above, it is possible to develop the fertilized eggs of Gallus domesticus repeatedly in vitro to a point where a large vascular area and an embryo can be seen. The longest time in which it was possible to maintain development was about five days. The embryo thus produced showed typical spontaneous movements. But in-vitro development, thus far, has always resulted in rupture of the yolk membrane—a disadvantageous circumstance due to an increase in volume of the egg. This hernia formation can also occur under normal conditions of development.

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PLATE 1

EXPLANATION OF FIGURES

1 A hen's egg, twenty-two hours in vitro. Note germinal disc, yellow yolk, albumen (with two chalazae), and outermost zone of salt solution containing air bubbles.

2 The same egg, forty-eight hours in vitro. Note ring-shaped blastoderm, rapidly increasing in size, and formation of rents in yolk mass.

3 A chick embryo, four days in vitro. Note constriction of yolk into two parts, the upper half of which (carrying the embryo) is again subdivided into three sacculations.

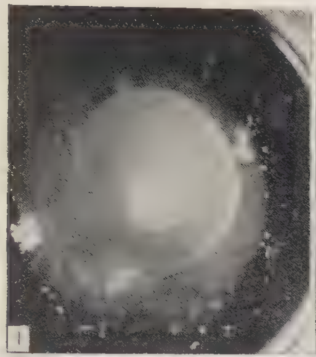
4 The same embryo, five days in vitro. The yolk mass is now constricted into two portions through coalescence of the three sacculations in the upper pole. Note differentiation of cerebral vesicles and limb buds. This embryo showed spontaneous movements.

Figures 3 and 4 were made with Hauff-Diapositiv plates, the others with Gevaert-Filtered-Orthochrom plates. That is the reason why the yolk in figures 3 and 4 takes black in these photographs and white in the others.

5 to 7 An embryo 92, 121, and 134 hours in vitro. Note formation of large hernia through rupture of yolk membrane. As the blastoderm is stretched, the blood islands are separated, so that no blood vessels are formed.

8 Normally incubated egg, showing a hernia of the yolk. The embryo with the whole vascular area lies on the hernia. Note curled edge of ruptured yolk membrane.

9 Normally incubated egg in which a hernia of the yolk has been produced by tearing the yolk membrane with a needle.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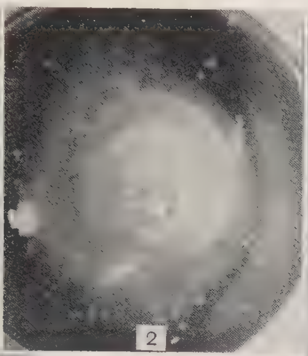
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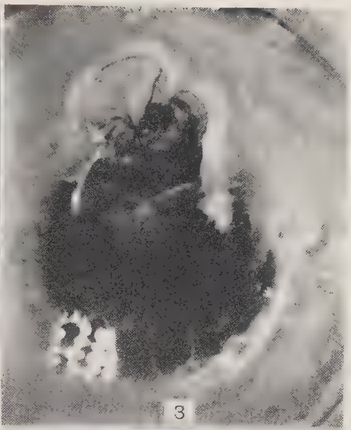
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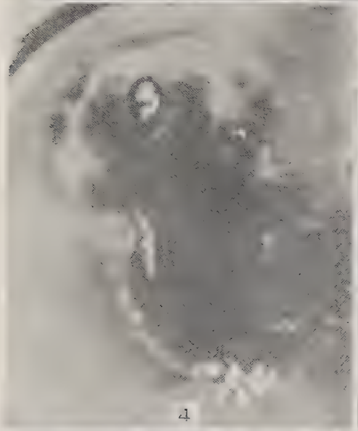
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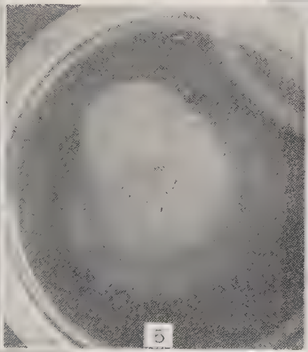
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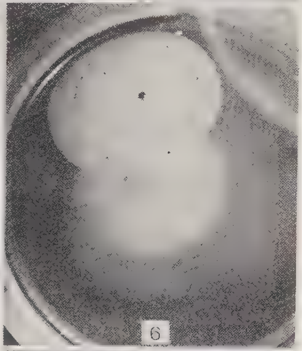
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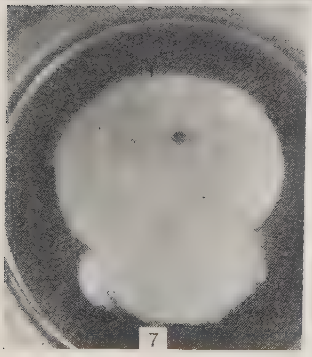
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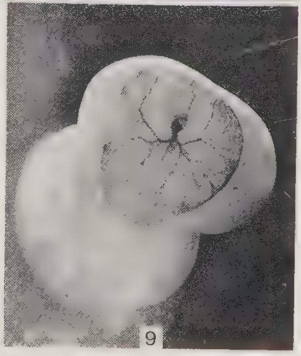
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